PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/82, 15/29, 9/02, A01H 5/00 — A1

(11) International Publication Number: WO 99/20775

(43) International Publication Date: 29 April 1999 (29.04.99)

(21) International Application Number: PCT/EP98/06978

(22) International Filing Date: 20 October 1998 (20.10.98)

(30) Priority Data:
08/956,182 22 October 1997 (22.10.97) US

(71) Applicant: RHONE-POULENC AGRO [FR/FR]; 14/20, rue Pierre Baizet, F-69009 Lyon (FR).

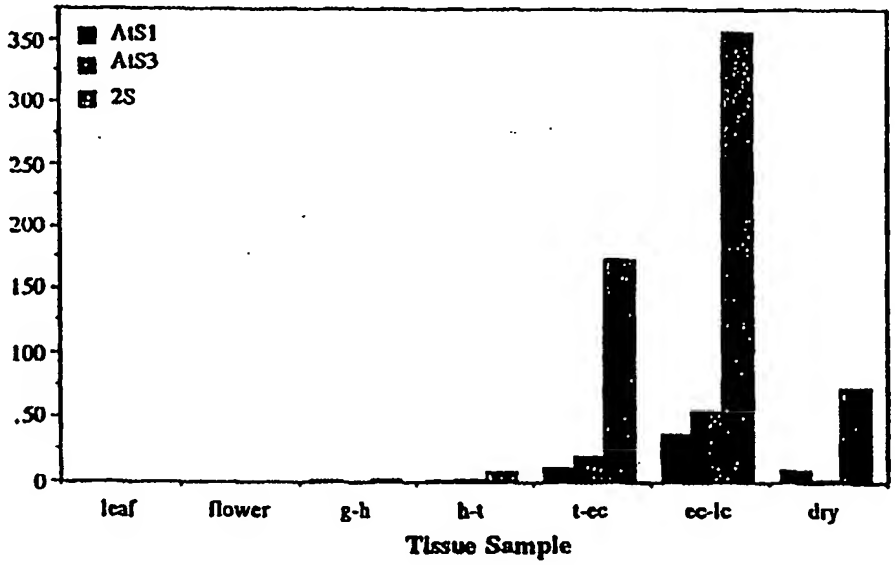
(72) Inventors: NUCCIO, Michael; P.O. Box 553, College Stations, TX 77841 (US). THOMAS, Terry; 2804 Chemin Drive, College Stations, TX 77845 (US).

(74) Agent: TETAZ, Franck; Rhône-Poulenc Agro – DPI, 14/20, rue Pierre Baizet, Boîte postale 9163, F-69263 Lyon Cedex 09 (FR).

(81) Designated States: AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IS, JP, KP, KR, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NOVEL SEED SPECIFIC PROMOTERS BASED ON PLANT GENES



(57) Abstract

The present invention is directed to 5' regulatory regions of two *Arabidopsis* seed-specific genes, AtS1 and AtS3. The 5' regulatory regions, or parts thereof, when operably linked to either the coding sequence of a heterologous gene or a sequence complementary to a native plant gene, direct expression of the coding sequence or complementary sequence in a plant seed. The regulatory regions are useful in expression cassettes and expression vectors for the transformation of plants. Also provided are methods of modulating the levels of a heterologous gene such as a fatty acid synthesis or lipid metabolism gene by transforming a plant with the subject expression cassettes and expression vectors.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

NOVEL SEED SPECIFIC PROMOTERS BASED ON PLANT GENES

Promoter analysis of seed-specific genes has a rich history (reviewed in Goldberg et al. (1989) *Cell, 56*; 149-160; Thomas (1993) *Plant Cell, 5*; 1401-1410). This stems from the observation that no plant gene is more tightly regulated in terms of spatial expression than those encoding seed storage proteins. Many seed storage protein genes have been cloned from diverse plant species, and their promoters have been analyzed in detail (Thomas, 1993). In these experiments promoter elements, which constitute the 5'-upstream regulatory regions, were functionally defined by their ability to confer seed-specific expression of the bacterial b-glucuronidase (GUS) reporter gene in transgenic plants (Bogue et al. (1980) *Mol. Gen. Genet.. 222*; 49-57; Bustos et al. (1989) *Plant Cell, 1*; 839-853). Results of this work initiated efforts to functionally define *cis*-elements to these genes that are critical for conferring seed-specific expression.

Later experiments involved construction of deletion mutants consisting of target promoters fused to the GUS-reporter gene. Analysis of these constructs in transgenic plants allowed researchers to define regions within each promoter that are critical to its overall regulation (Bustos et al. (1991) *EMBO J., 10*; 1469-1479; Chung (1995) *Ph.D. Dissertation. Texas A&M University*; Nunberg et al. (1994) *Plant Cell, 6*; 473-486). A general conclusion from this work is that the promoter proximal region contributes primarily to the gene's tissue specificity with more distal regions being responsible for modulating expression levels (Thomas, 1993). In addition to this, several groups have identified and characterized specific *cis*-regulatory elements, in both the promoter proximal region (PPR) and more distal regions, which interact with DNA binding proteins (Bustos et al., 1989; Chung, 1995; Jordano et al. (1989) *Plant Cell, 1*; 855-866; Nunberg et al., 1994). The functional significance of these regulatory elements varies from gene to gene.

In some cases, *cis*-regulatory elements have been mapped and the *trans*-acting factors which confer functionality have been cloned. For example, elements that allow the wheat EM-gene to respond to the plant hormone abcisisic acid (ABA) have been defined. This work led to the identification of a DNA binding protein which mediates this response (Guiltinan et al. (1990) *Science. 250*: 267-271; Marcotte et al.

(1989) *Plant Cell. 1*: 969-976). Putative ABA responsive elements have also been mapped in the sunflower helianthinin promoter HaG3-D and the carrot Dc3 promoter (Chung, 1995; Nunberg et al., 1994). Alone these elements act as positive elements in response to ABA. Regulation is restricted to the embryo, however, in the presence of each gene's promoter proximal region (Thomas, 1993).

Despite considerable effort, the *cis*-regulatory elements which contribute to a promoter's seed-specificity remain elusive (Chung, 1995; Li (1995) *Ph.D. Dissertation, Texas A&M University*). Recent work on the carrot Dc3 promoter proximal region has identified two bZIP genes that functionally interact with critical *cis*-elements (Kim et al. (1997) *Plant J., 11:* 1237-1251). This work has increased the understanding of seed-specific gene expression but it has also revealed that seed-specific gene regulation is complex.

In *Arabidopsis thaliana*, the promoters driving the expression of four members of the 2S albumin gene family have been analyzed in detail. The data indicate that each promoter is capable of conferring seed specific expression of a reporter gene in transgenic plants. Each promoter, however, confers slightly different spatial accumulation of the reporter in the developing seed. Thus, each family member contributes to the overall accumulation of the 2S albumins in the developing embryo. This is not unusual behavior for small gene families in plants (Lam et al. (1995) *Plant Cell, 7*; 887-898; Conceicao et al. (1994) *Plant J., 5*; 493-505; Sjödahl et al. (1993) *Plant Mol. Biol., 23*; 1165-1176; Pang et al. (1988) *Plant Mol. Biol., 11*; 805-820). In such cases, each member is capable of functionally complementing the others. The expression of each member is under different regulatory control leading to unique expression patterns. This appears to be a widespread gene regulatory mechanism in plants.

Little information is available on the contribution of a gene's untranslated elements to overall gene activity. In particular, the role of a gene's 5'-transcribed but untranslated region has never been fully investigated and is therefore not well understood. It is clear from the analysis of several plant genes, that these regions can significantly contribute to overall gene activity (Fu et al. (1995b) *Plant Cell, 7*; 1395-1403; Larkin et al. (1993) *Plant Cell, 5*; 1739-1748; Sieburth et al. (1997) *Plant*

Cell. 9; 355-365). The general role of these regions, if any, is not known. This is mainly due to the observation that a gene's promoter, defined as the gene's 5'-untranscribed region which consists of 1.0-1.5 kb of 5'-upstream sequence, is necessary and sufficient to confer spatial and temporal expression of the GUS reporter gene in transgenic plants. It may or may not be sufficient to account for overall gene activity. A general comparison of these regions reveals little or no conservation between diverse genes, and a similar observation has been made with respect to promoter elements as well (Conceicao et al., 1994).

Despite the uncertainties associated with seed-specific regulatory elements, there is substantial interest in identification and isolation of such regulatory elements for use in manipulating expression of both native and heterologous genes in plant seeds. For example, well-defined seed specific regulatory elements are useful in manipulating fatty acid synthesis or lipid metabolism genes in plant seeds. Other important agronomic traits such as herbicide and pesticide resistance, and drought tolerance may also be altered in the plant seed by transforming plants with appropriate heterologous genes under the control of well-defined seed-specific promoters and *cis* regulatory elements.

The present invention provides regulatory elements including promoters and 5' untranslated regions from two seed-specific plant genes designated AtS1 and AtS2. The regulatory elements may be used with any native or heterologous gene or portion thereof for expression of a corresponding gene product in a plant seed.

The present invention is directed to 5' regulatory regions of two seed-specific plant genes, AtS1 and AtS3.

In one embodiment this invention is directed to isolated nucleic acids comprising AtS1 5' regulatory regions which direct seed-specific expression including AtS1 promoters.

In another embodiment the present invention is directed to isolated nucleic acids comprising AtS3 regulatory regions which direct seed-specific expression including AtS3 promoters.

In a further embodiment the present invention is directed to vectors containing the isolated nucleic acids constituting the 5' regulatory regions of AtS1 and

AtS3, respectively.

In still another embodiment, this invention is drawn to plants transformed with the vectors containing the isolated nucleic acids constituting the 5' regulatory regions of AtS1 and AtS3, respectively, including the progeny generated from such transformed plants.

In another embodiment, the present invention is drawn to a transgenic plant comprising the isolated nucleic acid which constitutes the 5' regulatory region of AtS1 and AtS3, respectively.

In still a further embodiment this invention contemplates expression cassettes which comprise AtS1 5' regulatory regions including promoters operably linked to a heterologous gene or a nucleic acid encoding a sequence complementary to the native plant gene and vectors containing such expression cassettes. In another embodiment, the present invention is directed to expression cassettes which comprise AtS3 5' regulatory regions including promoters operably linked to a heterologous gene or nucleic acid encoding a sequence complementary to the native plant gene and vectors containing such expression cassettes.

In one embodiment this invention contemplates a method for directing seed-specific expression in a plant by providing such plant with an isolated nucleic acid comprising an AtS1 or AtS3 5' regulatory region to effect such seed-specific expression.

The present invention provides an isolated nucleic acid comprising a 5' regulatory region from a plant gene which direct seed specific expression, wherein the gene is selected from the group consisting in an AtS1 gene or an AtS3 gene.

As used herein, the term «regulatory region» can be further defined as comprising a promoter as well as 5' untranslated regions.

In another embodiment of the invention, there is provided an isolated nucleic acid comprising a promoter from a plant gene which direct seed specific expression, characterized in that the gene is selected from the group consisting in an AtS1 gene or an AtS3 gene.

The promoter of both the AtS1 and AtS2 gene is defined as the gene's 5' untranscribed region, generally consisting of 1.0 to 1.5 kb of 5' upstream sequence.

In another embodiment of the invention, there is provided an isolated

nucleic acid comprising a 5' transcribed and untranslated region from a plant gene which directs seed specific expression, characterized in that the gene is selected from the group consisting in an AtS1 gene or an AtS3 gene.

The 5' transcribed but untranslated region, is located immediately downstream from the promoter and ends just prior to the translational start of the AtS1 or AtS3 gene.

The term «seed-specific expression» as used herein, refers to expression in various portions of a plant seed such as the endosperm and embryo.

As a preferred embodiment, the plant is Arabidopsis.

The isolated nucleic acid of the invention is useful in the construction of expressions cassettes which comprises in the 5' to 3' direction, an isolated nucleic acid of the invention, a heterologous gene or sequence complementary to a native plant gene and a 3' termination sequence. Such an expression cassette can be incorporated in a variety of autonomously replicating vectors in order to construct an expression vector.

As used herein, the term «cassette» refers to a nucleotide sequence capable of expressing a particular gene if said gene is inserted so as to be operably linked to one or more regulatory regions present in the nucleotide sequence. Thus, for example, the expression cassette may comprise a heterologous coding sequence which is desired to be expressed in a plant seed. The expression cassettes and expression vectors of the present invention are therefore useful for directing seed-specific expression of any number of heterologous genes.

In another embodiment of the invention, there is provided an expression cassette which comprises at least one 5' regulatory region of the invention, operably linked to at least one of a nucleic acid encoding a heterologous gene or a nucleic acid encoding a sequence complementary to a native plant gene.

In another embodiment of the invention, there is provided an expression cassette which comprises at least one promoter of the invention, operably linked to at least one of a nucleic acid encoding a heterologous gene or a nucleic acid encoding a sequence complementary to a native plant gene.

In another embodiment of the invention, there is provided an expression cassette which comprises at least one 5' transcribed and untranslated region of the

invention, operably linked at its 5' end to a promoter which functions in plants and operably linked at its 3' end to at least one of a nucleic acid encoding a heterologous gene or a nucleic acid encoding a sequence complementary to a native plant gene.

The present invention also provides a vector, a cell, a plant, progeny of the plant and seeds of the plant, which comprises an isolated nucleic acid and/or an expression cassette of the invention.

Figure 1 is a graph depicting developmental expression of three seed-specific *Arabidopsis* genes, AtS1, AtS3, and 2S. Abbreviations are as follows:
g-h, globular to heart stage siliques; h-t, heart to torpedo stage siliques; t-ec: torpedo to early cotyledon stage siliques; ec-lc, early cotyledon to late cotyledon stage siliques; dry, dry seed.

Figure 2A depicts an autoradiograph of the reaction products from differential display PCR amplifications resolved on a 6% sequencing gel. The arrow indicates the AtS1 gene.

Figure 2B depicts an autoradiograph of the reaction products from differential display PCR amplifications resolved on a 6% sequencing gel. The arrow indicates the AtS3 gene.

Figure 3A depicts an autoradiograph of an RNA gel blot probed with cDNA inserts representing the AtS1 gene. Abbreviations are as follows: F, flower; L, leaf; R, root; S, immature seed; Si, silique without seed. The Location of 28S and 18S ribosomal RNAs are indicated.

Figure 3A depicts an autoradiograph of an RNA gel blot probed with cDNA inserts representing the AtS1 gene. Abbreviations are as follows: F, flower; L, leaf; R, root; S, immature seed; Si, silique without seed. The Location of 28S and 18S ribosomal RNAs are indicated.

Figure 3B depicts an autoradiograph of an RNA gel blot probed with cDNA inserts representing the AtS3 gene. Abbreviations are as in Fig. 3A. The Location of 28S and 18S ribosomal RNAs are indicated.

Figure 4A shows alignment of the 3'-termini for six different AtS1 cDNAs, 1-1 (SEQ ID NO:1), 1-2 (SEQ ID NO:2), 1-3 (SEQ ID NO:3), 1-4 (SEQ ID NO:4), 1-5 (SEQ ID NO:5), and 1-6 (SEQ ID NO:6). The location of the poly(A) tail

on each cDNA is indicated by «An».

Figure 4B shows alignment of the 3'-termini for six different AtS3 cDNAs, 3-1 (SEQ ID NO:7), 3-2 (SEQ ID NO:8), 3-3 (SEQ ID NO:9), 3-4 (SEQ ID NO:10), 3-5 (SEQ ID NO:11), and 3-6 (SEQ ID N0:12). The location of the poly(A) tail on each cDNA is indicated by «An».

Figure 5A is a photomicrograph showing *in situ* localization of AtS1 mRNA in a globular stage embryo.

Figure 5B is a photomicrograph showing *in situ* localization of AtS1 mRNA in a heart stage embryo.

Figure 5C is a photomicrograph showing *in situ* localization of AtS1 mRNA in a early cotyledon stge embryo.

Figure 5D is a photomicrograph showing *in situ* localization of AtS1 mRNA in a late cotyledon stage embryo, cross section. The protoderm (P) and provasculature (V) are indicated by the arrows.

Figure 5E is also a photomicrograph showing *in situ* localization of AtS1 mRNA in a late cotyledon stage embryo, cross section.

Figure 5F is a photomicrograph showing *in situ* localization of AtS1 mRNA in a late cotyledon stage embryo, longitudinal section.

Figure 6A is a photomicrograph showing *in situ* localization of AtS3 mRNA in an early cotyledon stage embryo.

Figures 6B and 6C are photomicrographs showing *in situ* localization of AtS3 mRNA in early cotyledon stage embryos, cross sections.

Figures 6D, 6E, and 6F are photomicrographs showing *in situ* localization of AtS3 mRNA in an late cotyledon stage embryos, longitudinal sections.

Figure 7A shows two southern hybridizations of *Arabidopsis* genomic DNA probed with either AtS1 or AtS3 cDNA probes under high stringency conditions. The arrows on the right indicate the genomic fragments that were subcloned for sequence analysis. Abbreviations are as follows: B,*Bam HI*; E,*EcoRI*; H,*HindIII*; S,*SacI*; X, *XbaI*.

Figure 7B shows two southern hybridizations of *Arabidopsis* genomic DNA probed with either AtS1 or AtS3 cDNA probes under low stringency conditions.

Abbreviations are as in Figure 7A.

Figure 8 depicts the nucleotide sequence of a portion of a 5.5 kb genomic fragment containing the AtS1 gene (SEQ ID NO:13). The portion of the 5.5 kb fragment which aligns with the AtS1 cDNA and putative AtS1 protein (indicated in italics) is shown as well as sequence upstream from the translational start site and downstream from the translational stop. Two transcription start sites were mapped and are indicated by the double underline. The location of several polyadenylation sites are marked by the asterisks. The location of a putative CAAT box and TFID binding site are underlined.

Figure 9 depicts the nucleotide sequence of a portion of a 7.9 kb genomic fragment containing the AtS3 gene (SEQ ID NO:14). The portion of the 7.9 kb fragment which aligns with the AtS1 cDNA and putative AtS1 protein (indicated in italics) is shown as well as sequence upstream from the translational start site and downstream from the translational stop. Four transcription start sites were mapped and are indicated by the double underline. The location of several polyadenylation sites are marked by the asterisks. The location of a putative CAAT box and TFID binding site are underlined.

Figure 10A is an autoradiograph of the reaction products of an RNAase protection assay electrophoresed through a 6% sequencing gel and used to identify the transcriptional start site for the AtS1 gene. Protected fragments were identified as bands (indicated by arrows) which increase in intensity as total RNA template increases. Bases corresponding to these protected fragments are indicated by a double under line in Figure 8.

Figure 10B is an autoradiograph of the reaction products of an RNAase protection assay electrophoresed through a 6% sequencing gel and used to identify the transcriptional start site for the AtS3 gene. Protected fragments were identified as bands (indicated by arrows) which increase in intensity as total RNA template increases. Bases corresponding to these protected fragments are indicated by a double under line in Figure 9.

Figure 11A shows organization of the AtS1 genomic clone. The direction of transcription is indicated by the arrows and additional transcribed regions

are also indicated. Exons are depicted by gray blocks, introns and non-coding sequences by lines, translational start sites by arrows and translational stop sites by a bar.

Figure 11B shows organization of the AtS3 genomic clone. The direction of transcription is indicated by the arrows and additional transcribed regions are also indicated. Exons, introns and non-coding sequences are as depicted in Figure 11A.

Figure 12A depicts a western blot of *Arabidopsis* total protein (P) and developing silique protein (S) reacted against rabbit antisera raised against fusion proteins representing the AtS1 gene product. The reaction was detected using an anti-rabbit antibody conjugated to alkaline phosphatase.

Figure 12B depicts a western blot of *Arabidopsis* total protein (P) and developing silique protein (S) reacted against rabbit antisera raised against fusion proteins representing the AtS3 gene product. The reaction was detected using an anti-rabbit antibody conjugated to alkaline phosphatase.

Figure 13A depicts immunolocalizaton of the AtS1 gene product in an immature seed. The fusion proteins were raised in *E. coli* and affinity purified prior to injection into rabbits. The reaction was detected using an anti-rabbit antibody conjugated to alkaline phosphatase.

Figure 13B depicts immunolocalization of the AtS3 gene product in an immature seed. Fusion proteins were raised as in Fig. 13A and hybridization was detected as in Fig. 13A.

Figure 14 shows the chromosome map position of AtS1 by RFLP analysis.

Figure 15A shows the alignment of the AtS1 (SEQ ID NO:15) and EFA27 (SEQ ID NO:16) cDNAs using the FASTA algorithm.

Figure 15B shows the alignment of the AtS1 (SEQ ID NO:17) and EFA27 (SEQ ID NO:18) gene products using the PIR algorithm (Huang et al. (1991) *Advances in Applied Mathematics, 12*; 337-357) Asterisks indicate identity.

Figure 16A shows alignment of the AtS1 (SEQ ID NO:19) coding sequence with the sequence of the expressed sequence tag clone ATTS0251

(ATTS)(SEQ ID NO:20) using the FASTA algorithm.

Figure 16B shows alignment of the EFA27 coding sequence (SEQ ID NO:21) with the sequence of the expressed sequence tag clone ATTS0251 (ATTS)(SEQ ID NO:22) using the FASTA algorithm.

Figure 17A is a graph depicting hydropathy analysis for AtS1. The conceptual open reading frame for AtS1 was translated and subjected to Kyte Doolittle hydropathy analysis algorithm.

Figure 17B is a graph depicting hydropathy analysis for AtS2. The conceptual open reading frame for AtS3 was translated and subjected to Kyte Doolittle hydropathy analysis algorithm.

Figure 18A illustrates AtS1:GUS fusions. The construct denoted «tsp» represent transcriptional fusions; those denoted «tlp» represent translational fusions. The AtS1 genomic clone is pictured above the AtS1:GUS fusions to illustrate the elements included in each construct.

Figure 18B illustrates AtS3:GUS fusions. Transcriptional and translational fusions are designated «tsp» and «tlp», respectively. The AtS3 genomic clone is pictured above the AtS3:GUS fusions to illustrate the elements included in each construct.

Figure 19A graphically depicts developmental expression of the AtS1 and AtS3 transcriptional fusions, 1tsp and 3tsp in transgenic *Arabidopsis*. Abbreviations are as follows: l, leaf; g-t, globular to torpedo stage embryos; ec, early cotyledon embryos; lc, late cotyledon embryos; and dry, mature dry seeds. Each tissue sample was assayed in triplicate and the data represents the mean between individual plants.

Figure 19B graphically depicts developmental expression of the AtS1 and AtS3 translational fusions, 1tlp and 3tlp in transgenic *Arabidopsis*. Abbreviations are as in Figure 19A. Each tissue sample was assayed in triplicate and the data represents the mean between individual plants.

Figure 20A shows histochemical localization of GUS activity in a mature *Arabidopsis* embryo from a 1tsp transgenic line.

Figure 20B shows histochemical localization of GUS activity in a mature

Arabidopsis embryo from a 1tlp transgenic line.

Figure 20C shows histochemical localization of GUS activity in a mature *Arabidopsis* embryo from a 3tsp transgenic line.

Figure 20D shows histochemical localization of GUS activity in a mature *Arabidopsis* embryo from a 3tlp transgenic line.

Figure 21A graphically depicts developmental expression of the AtS1 and AtS2 transcriptional promoter:GUS fusions in transgenic tobacco. «L» denotes leaf tissue; the remaining bars denote developing seeds representing 5, 10, 15, 20, 25 and 30 days post flowering (DPF). Each tissue sample was assayed in triplicate and the data represents the mean between individual plants. The data represents the average of at least two individuals.

Figure 21B graphically depicts developmental expression of the AtS1 and AtS2 translational promoter:GUS fusions in transgenic tobacco. «L» denotes leaf tissue; the remaining bars denote developing seeds representing 5, 10, 15, 20, 25 and 30 days post flowering (DPF). Each tissue sample was assayed in triplicate and the data represents the mean between individual plants. The data represents the average of at least two individuals.

Figure 22A shows histochemical localization of GUS activity in a mature tobacco embryo from a 1tsp transgenic line.

Figure 22B shows histochemical localization of GUS activity in a mature tobacco embryo from a 1tlp transgenic line.

Figure 22C shows histochemical localization of GUS activity in a mature tobacco embryo from a 3tsp transgenic line.

Figure 22D shows histochemical localization of GUS activity in a mature tobacco embryo from a 3tlp transgenic line.

Figure 23 shows the nucleotide sequence of the 1tsp promoter element. The promoter is derived from the AtS1 gene. The promoter element was amplified by Pfu polymerase. The amplified promoter was cloned into the HindIII/BamHI sites in the vector pBI121 as a SacI/BamHI fragment. At the 5'-end the lower case sequence is what remains of the HindIII site and the SacI site (AtS1 promoter). The putative transcription site is indicated by a +1. Non-AtS1 spacer sequence is shown in italics;

sequence to the right of the double underlined region is derived from the pBI121 polylinker (SEQ ID NO:39).

Figure 24 shows the structure of the 1tlp promoter element. The promoter is derived from the AtS1 gene (genomic clone ddp5g in pBluescript as a SacI fragment). The promoter element was amplified by Pfu polymerase. It was initially cloned into the vector NCO-GUS as a PstI/NcoI fragment. The promoter: GUS fusion was moved into pBIN19 as a BAMHI/EcoRI fragment. The sequence shown is the promoter element itself as sequenced from the expression cassette. The BamHI site (5') and NcoI site (3') are in bold. The 5'-UTL is underlined, and the putative transcriptional start site is indicated by +1. The SacI site at the 5'-terminus is also underlined. This signifies the 5'-terminus of the AtS1 gene. The sequence preceding it is derived from the cloning vectors used to construct this expression cassette. The translation start site is double underlined. (SEQ ID NO:40).

Figure 25 shows the structure of the 3tsp promoter element. The promoter is derived from the AtS3 gene (genomic clone ddp8g in pBluescript as a XbaI fragment). The promoter element was amplified by Pfu polymerase. It was initially cloned into the vector pBI101 as a XbaI/blunt fragment. The sequence shown is the promoter element itself as sequenced form the expression cassette. The 5' XbaI site is in bold. The presumed transcriptional start site is designated as +1. The underlined sequence represents non-AtS3 spacer sequence. This region includes a BamHI site (underlined and in bold); this site was originally engineered into the primer used to amplify the promoter element but was not used in the cloning procedure. Nucleotides 3' of this BamHI site are from the PBI101 polylinker region. (SEQ ID NO:41).

Figure 26 shows the structure of the 3tlp promoter element. The promoter is derived from the AtS3 gene (genomic clone ddp8g in pBluescript as an XbaI fragment). The promoter element was amplified by Pfu polymerase. The amplified promoter was initially cloned into the vector NCO-GUS as an XbaI/NcoI fragment. The promoter::GUS fusion was moved into pBIN19 as an XbaI/EcoRI fragment. The sequence shown is the promoter element itself as sequenced from the expression cassette. The XbaI site (5') and NcoI sites (3') are in bold. The 5'-UTL is underlined. The translation start site is double-underlined. (SEQ ID NO:42).

An isolated nucleic acid encoding a 5' regulatory region from an *Arabidopsis* AtS1 gene can be provided as follows. AtS1 recombinant genomic clones are first isolated by screening a plant genomic DNA library with a cDNA (or a portion thereof) representing AtS1 mRNA. An expressed sequence tag (EST) representing the AtS1 gene has been identified in an *Arabidopsis* dry seed library. The GeneBank accession numbers for the *est* clone (cDNA number pap232) are Z2053 and Z29900.

Methods considered useful in obtaining genomic recombinant DNA sequences corresponding to the AtS1 gene of the present by screening a genomic library are provided in Sambrook et al. (1989), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, for example, or any of the myriad of laboratory manuals on recombinant DNA technology that are widely available.

An isolated nucleic acid encoding a 5' regulatory region from an *Arabidopsis* AtS1 or AtS3 gene can also be identified using an improved differential display method, described in detail herein. The differential display method is a PCR based technology which is designed to subdivide an mRNA population into reasonably comparable groups. This improved methodology permits matching the TmS of the random primer and the oligo dT primers. Rather than using internal labeling to ensure the dT primer is included in the reaction and increasing the signal or noise the improved process permits labeling the oligo dT primers. In accordance with the present invention, instead of cloning candidate differential display products, the products were used as probes to screen full length cDNA libraries. PCR-based RNA fingerprinting is used to directly compare the expression of arbitrary genes from many tissues, allowing the identification of uniquely expressed genes.

The present invention also provides for an improved differential display gene isolation method than that of the prior art e.g., Liang et al. (1992) *Science, 257*; 967-971. The improved method employs accurate amplification, i.e., a mechanism to ensure that the oligonucleotide primers used for the analysis are functioning properly.

For example, by reducing the mRNA complexity, individual mRNAs may be accurately compared. This reduction is initially achieved by selectively priming cDNA synthesis with an anchored oligo-dT-primer. Although the primer needs to participate in both the cDNA synthesis and the PCR amplification step, the methods

of the prior art do not effectively prime DNA synthesis since the annealing temperatures are too high. As a result, although the primer is designed to designate the mRNA population to be analyzed, differential display products having the primer are difficult to identify.

By lowering the annealing temperature as provided by the present invention, selecting for differential display products which contain the primer increase the likelihood that they in fact represent *bona fide* targets. Lowering the annealing temperature, however, also increases the background associated with differential display. Since the primer is more efficient in the PCR amplification step, reaction products containing just the primer will likely be very abundant and are therefore removed.

Stringent selection is also an important element in the improved differential display process of the present invention. A stringent mechanism to remove the background hybridization is required to avoid screening through each cDNA clone individually. For example, a differential display band likely represents more than one DNA template and the signal sequence needs to be purified away from the background sequences. In isolating an AtS1 or AtS3 gene, the cDNA library represents poly(A)-enriched RNA made from mRNAs isolated from seeds. Screening the library under high stringency conditions should select against background sequences including cDNAs generated from tRNA or rRNA templates.

Exemplification of the differential display analysis in isolating AtS1 and AtS3 seed-specific genes is given in Example 1.

To determine nucleotide sequences, a multitude of techniques are available and known to the ordinarily skilled artisan. For example, restriction fragments containing a corresponding AtS1 or AtS3 regulatory region can be subcloned into the polylinker site of a sequencing vector such as pBluescript (Stratagene). These pBluescript subclones can then be sequenced by the double-stranded dideoxy method (Chen et al. (1985) *DNA, 4*; 165).

In a preferred embodiment of the present invention, the AtS1 promoter comprises nucleotides 6-1216 of Fig. 23 (SEQ ID NO:23). The AtS3 promoter preferably comprises nucleotides 7-1486 of Fig. 25 (SEQ ID NO:24). In another

preferred embodiment, the AtS1 5' transcribed and untranslated region comprises nucleotides 1326 to 1387 of Fig. 24 (SEQ ID NO:25). In yet another preferred embodiment, the AtS3 5' transcribed and untranslated region comprises nucleotides 1472 to 1537 of Fig. 26 (SEQ ID NO:26).

In a more preferred embodiment, the AtS1 regulatory region is made up of both the promoter and 5' transcribed and untranslated region and comprises nucleotides 42 to 1387 of Fig. 24 (SEQ ID NO:27). In another more preferred embodiment, the AtS3 regulatory region is made up of both the promoter and 5' transcribed but untranslated region and comprises nucleotides 7 to 1537 of Fig. 26 (SEQ ID NO:28).

Modifications to the AtS1 and AtS3 regulatory regions, including the individual promoters and 5' transcribed but untranslated regions as set forth in SEQ ID NOS:23 through 28, which maintain the characteristic property of directing seed-specific expression, are within the scope of the present invention. Such modifications include insertions, deletions and substitutions of one or more nucleotides.

The subject AtS1 and AtS3 5' regulatory regions and parts thereof such as promoters and 5' transcribed but untranslated regions, can be derived from restriction endonuclease or exonuclease digestion of isolated AtS1 or AtS3 genomic clones. Thus, for example, the known nucleotide or amino acid sequence of the coding region of an isolated AtS1 or AtS3 gene (e.g. Figs. 8 and 9) is aligned to the nucleic acid or deduced amino acid sequence of an isolated seed-specific genomic clone and the 5' flanking sequence (i.e., sequence upstream from the translational start codon of the coding region) of the isolated AtS1 and AtS3 genomic clone located.

The AtS1 and AtS3 5' regulatory regions as set forth in SEQ ID NOs: 27 and 28 respectively, (nucleotides 42 to 1387 of Fig. 24 and nucleotides 7-1537 of Fig. 26, respectively) may be generated from genomic clones having either or both excess 5' flanking sequence or coding sequence by exonuclease III-mediated deletion. This is accomplished by digesting appropriately prepared DNA with exonuclease III (exoIII) and removing aliquots at increasing intervals of time during the digestion. The resulting successively smaller fragments of DNA may be sequenced to determine the exact endpoint of the deletions. There are several commercially available systems which use

exonuclease III (exoIII) to create such a deletion series. e.g. Promega Biotech. «Erase-A-Base» system. Alternatively, PCR primers can be defined to allow direct amplification of the subject AtS1 or AtS3 regulatory regions, or parts thereof such as promoters and 5' transcribed but untranslated regions.

Using the same methodologies, the ordinarily skilled artisan can generate one or more deletion fragments of the regulatory regions of the AtS1 and AtS2 genes as set forth in SEQ ID NOs: 27 and 28 respectively. Any and all deletion fragments which comprise a contiguous portion of the nucleotide sequences set forth in any of SEQ ID NOS:23, 24, 25, 26, 27, or 28 and which retain the capacity to direct seed-specific expression are contemplated by the present invention.

Confirmation of seed-specific 5' regulatory regions which direct seed-specific expression and modifications or deletion fragments thereof, can be accomplished by construction of transcriptional and/or translational fusions of specific sequences with the coding sequences of a heterologous gene, transfer of the chimeric gene into an appropriate host, and detection of the expression of the heterologous gene. The assay used to detect expression depends upon the nature of the heterologous sequence. For example, reporter genes, exemplified by chloramphenicol acetyl transferase and b-glucuronidase (GUS), are commonly used to assess transcriptional and translational competence of chimeric constructions. Standard assays are available to sensitively detect the reporter enzyme in a transgenic organism. The b-glucuronidase (GUS) gene is useful as a reporter of promoter activity in transgenic plants because of the high stability of the enzyme in plant cells, the lack of intrinsic b-glucuronidase activity in higher plants and availability of a quantitative fluorimetric assay and a histochemical localization technique. Jefferson et al. (1987b) *EMBO J 6;* 3901-3907 have established standard procedures for biochemical and histochemical detection of GUS activity in plant tissues. Biochemical assays are performed by mixing plant tissue lysates with 4-methylumbelliferyl-b-D-glucuronide, a fluorimetric substrate for GUS, incubating one hour at 37°C, and then measuring the fluorescence of the resulting 4-methyl-umbelliferone. Histochemical localization for GUS activity is determined by incubating plant tissue samples in 5-bromo-4-chloro-3-indolyl-glucuronide (X-Gluc) for about 18 hours at 37°C and observing the staining pattern of X-Gluc. The construction

of such chimeric genes allows definition of specific regulatory sequences and demonstrates that these sequences can direct expression of heterologous genes in a seed-specific manner.

Another aspect of the invention is directed to expression cassettes and expression vectors (also termed herein «chimeric genes») comprising a 5' regulatory region or portion thereof from an AtS1 or AtS3 gene which direct seed specific expression operably linked to the coding sequence of a heterologous gene such that the regulatory element is capable of controlling expression of the product encoded by the heterologous gene. The heterologous gene can be any gene other than AtS1 or AtS3. If necessary, additional regulatory elements from genes other than AtS1 or AtS3 or parts of such elements sufficient to cause expression resulting in production of an effective amount of the polypeptide encoded by the heterologous gene are included in the chimeric constructs.

Accordingly, the present invention provides chimeric genes comprising sequences of the AtS1 or AtS3 5' regulatory region that confer seed-specific expression which are operably linked to a sequence encoding a heterologous gene such as a lipid metabolism enzyme. Examples of lipid metabolism genes useful for practicing the present invention include lipid desaturases such as D6-desaturases, D12-desaturases, D15-desaturases and other related desaturases such as stearoyl-ACP desaturases, acyl carrier proteins (ACPs), thioesterases, acetyl transacylases, acetyl-coA carboxylases, ketoacyl-synthases, malonyl transacylases, and elongases. Such lipid metabolism genes have been isolated and characterized from a number of different bacteria and plant species. Their nucleotide coding sequences as well as methods of isolating such coding sequences are disclosed in the published literature and are widely available to those of skill in the art.

In particular, the D6-desaturase genes disclosed in U.S. Patent Nos. 5,552,306 and 5,614,393 and incorporated herein by reference, are contemplated as lipid metabolism genes particularly useful in the practice of the present invention.

The chimeric genes of the present invention are constructed by ligating a 5' regulatory region or part thereof, of an AtS1 or AtS3 genomic DNA to the coding sequence of a heterologous gene. The juxtaposition of these sequences can be

accomplished in a variety of ways. In one embodiment, the order of sequences in a 5' to 3' direction, is an AtS1 or AtS3 promoter, a coding sequence, and a termination sequence. In a preferred embodiment, the order of the sequences in a 5' to 3' direction is an AtS1 or AtS3 promoter, an AtS1 or AtS3 transcribed but untranslated region, a coding sequence, and a termination sequence which includes a polyadenylation site.

Standard techniques for construction of such chimeric genes are well known to those of ordinary skill in the art and can be found in references such as Sambrook et al.(1989). A variety of strategies are available for ligating fragments of DNA, the choice of which depends on the nature of the termini of the DNA fragments. One of ordinary skill in the art recognizes that in order for the heterologous gene to be expressed, the construction requires at least a promoter and signal for efficient polyadenylation of the transcript. Accordingly, the AtS1 or AtS3 5' regulatory region that contains the consensus promoter sequence known as the TATA box can be ligated directly to a promoterless heterologous coding sequence.

The restriction or deletion fragments that contain the AtS1 or AtS2 TATA box are ligated in a forward orientation to a promoterless heterologous gene such as the coding sequence of b-glucuronidase (GUS). The skilled artisan will recognize that the subject AtS1 or AtS3 5' regulatory regions and parts thereof, can be provided by other means, for example chemical or enzymatic synthesis.

The 3' end of a heterologous coding sequence is optionally ligated to a termination sequence comprising a polyadenylation site, exemplified by, but not limited to, the nopaline synthase polyadenylation site, or the octopine T-DNA gene 7 polyadenylation site. Alternatively, the polyadenylation site can be provided by the heterologous gene.

The present invention also provides methods of increasing levels of heterologous genes in plant seeds. In accordance with such methods, the subject expression cassettes and expression vectors are introduced into a plant in order to effect expression of a heterologous gene. For example, a method of producing a plant with increased levels of a product of a fatty acid synthesis or lipid metabolism gene is provided by transforming a plant cell with an expression vector comprising an AtS1 or AtS2 5' regulatory region or portion thereof, operably linked to a fatty acid synthesis or

lipid metabolism gene and regenerating a plant with increased levels of the product of said fatty acid synthesis or lipid metabolism gene.

Another aspect of the present invention provides methods of reducing levels of a product of a gene which is native to a plant which comprises transforming a plant cell with an expression vector comprising a subject AtS1 or AtS2 5' regulatory region or part thereof, operably linked to a nucleic acid sequence which is complementary to the native plant gene. In this manner, levels of endogenous product of the native plant gene are reduced through the mechanism known as antisense regulation. Thus, for example, levels of a product of a fatty acid synthesis gene or lipid metabolism gene are reduced by transforming a plant with an expression vector comprising a subject AtS1 or AtS3 5' regulatory region or part thereof, operably linked to a nucleic acid sequence which is complementary to a nucleic acid sequence coding for a native fatty acid synthesis or lipid metabolism gene.

The present invention also provides a method of cosuppressing a gene which is native to a plant which comprises transforming a plant cell with an expression vector comprising a subject 5' AtS1 or AtS3 regulatory region operably linked to a nucleic acid sequence coding for the native plant gene. In this manner, levels of endogenous product of the native plant gene are reduced through the mechanism known as cosuppression. Thus, for example, levels of a product of a fatty acid synthesis gene or lipid metabolism gene are reduced by transforming a plant with an expression vector comprising a subject AtS1 or AtS3 5' regulatory region or part thereof, operably linked to a nucleic acid sequence coding for a native fatty acid synthesis or lipid metabolism gene native to the plant. Although the exact mechanism of cosuppression is not completely understood, one skilled in the art is familiar with published works reporting the experimental conditions and results associated with cosuppression (Napoli et al. (1990) *The Plant Cell, 2:* 270-289; Van der Krol (1990) *Plant Mol. Biol, 14*; 457-466.)

To provide regulated expression of the heterologous or native genes, plants are transformed with the chimeric gene constructions of the invention. Methods of gene transfer are well known in the art. The chimeric genes can be introduced into plants by leaf disk transformation-regeneration procedure as described by Horsch et al. (1985) *Science, 227*; 1229-1231. Other methods of transformation such as protoplast

culture (Horsch et al. (1984) *Science, 223*; 496; DeBlock et al. (1984) *EMBO J., 2*; 2143; Barton et al. (1983) *Cell, 32*; 1033) can also be used and are within the scope of this invention. In a preferred embodiment, plants are transformed with *Agrobacterium*-derived vectors such as those described in Klett et al. (1987) *Annu. Rev. Plant Physiol., 38*; 467. Other well-known methods are available to insert the chimeric genes of the present invention into plant cells. Such alternative methods include biolistic approaches (Klein et al. (1987) *Nature, 327*; 70), electroporation, chemically-induced DNA uptake, and use of viruses or pollen as vectors.

When necessary for the transformation method, the chimeric genes of the present invention can be inserted into a plant transformation vector, e.g. the binary vector described by Bevan (1984) *Nucleic Acids Res., 12*; 8711-8721. Plant transformation vectors can be derived by modifying the natural gene transfer system of *Agrobacterium tumefaciens*. The natural system comprises large Ti (tumor-inducing)-plasmids containing a large segment, known as T-DNA, which is transferred to transformed plants. Another segment of the Ti plasmid, the *vir* region, is responsible for T-DNA transfer. The T-DNA region is bordered by terminal repeats. In the modified binary vectors, the tumor inducing genes have been deleted and the functions of the *vir* region are utilized to transfer foreign DNA bordered by the T-DNA border sequences. The T-region also contains a selectable marker for antibiotic resistance, and a multiple cloning site for inserting sequences for transfer. Such engineered strains are known as «disarmed» *A. tumefaciens* strains, and allow the efficient transfer of sequences bordered by the T-region into the nuclear genome of plants.

Surface-sterilized leaf disks and other susceptible tissues are inoculated with the «disarmed» foreign DNA-containing *A. tumefaciens*, cultured for a number of days, and then transferred to antibiotic-containing medium. Transformed shoots are then selected after rooting in medium containing the appropriate antibiotic, and transferred to soil. Transgenic plants are pollinated and seeds from these plants are collected and grown on antibiotic medium.

Expression of a heterologous or reporter gene in developing seeds, young seedlings and mature plants can be monitored by immunological, histochemical or activity assays. As discussed herein, the choice of an assay for expression of the

chimeric gene depends upon the nature of the heterologous coding region. For example, Northern analysis can be used to assess transcription if appropriate nucleotide probes are available. If antibodies to the polypeptide encoded by the heterologous gene are available, Western analysis and immunohistochemical localization can be used to assess the production and localization of the polypeptide. Depending upon the heterologous gene, appropriate biochemical assays can be used. For example, acetyltransferases are detected by measuring acetylation of a standard substrate. The expression of a lipid desaturase gene can be assayed by analysis of fatty acid methyl esters (FAMES).

Another aspect of the present invention provides transgenic plants or progeny of these plants containing the chimeric genes of the invention. Both monocotyledonous and dicotyledonous plants are contemplated. Plant cells are transformed with the chimeric genes by any of the plant transformation methods described above. The transformed plant cell, usually in the form of a callus culture, leaf disk, explant or whole plant (via the vacuum infiltration method of Bechtold et al. (1993) *C.R. Acad. Sci. Paris, 316*; 1194-1199) is regenerated into a complete transgenic plant by methods well-known to one of ordinary skill in the art (e.g., Horsh et al., 1985). In a preferred embodiment, the transgenic plant is sunflower, cotton, oil seed rape, maize, tobacco, *Arabidopsis*, peanut or soybean. Since progeny of transformed plants inherit the chimeric genes, seeds or cuttings from transformed plants are used to maintain the transgenic line.

The following examples further illustrate the invention.

EXAMPLE 1 IDENTIFICATION OF AtS1 and AtS3 AS SEED-SPECIFIC GENES

Both AtS1 and AtS3 were identified as seed-specific genes in *Arabidopsis* by differential display. The differential display method is a PCR based technology which is designed to subdivide an mRNA population into reasonably comparable groups. PCR-based RNA fingerprinting is used to directly compare the expression of arbitrary genes from many tissues, allowing the identification of uniquely expressed genes. (McClelland et al. (1995) *Trends Genet., 11*; 242-246; Nuccio et al. (1996) *SAAS Bulletin, Biochem. & Biotech., 9*; 23-28; Frugoli et al. (1996) *Heynh. Plant*

Physiol., *113*: 327-336; Vielle-Calzada et al. (1996) *Link. Plant Mol. Biol.*, *32*; 1085-1092).

Plant maintenance and tissue preparation

Arabidopsis thaliana (Landsberg) plants were grown under continuous illumination in a vermiculite/soil mixture at ambient temperature (22°C). Siliques were dissected 2 to 5 days after flowering to separate immature seeds from the silique coats. Both tissues were frozen in liquid nitrogen and stored at -85°C. Root tissue was obtained from elongated roots grown in liquid culture. The root cultures were started from 4 to 20 seeds which were surface sterilized with 10% bleach/0.1% SDS, rinsed thoroughly with water, and cultured in Gamborg B_5 medium for two weeks. Inflorescences containing initial flower buds and fully opened flowers, and leaves of different sizes were also collected.

RNA preparation

Total RNA was prepared following a procedure that has been modified from Galau et al. (1981) *J. Biol. Chem.*, *256*; 2551-2560 and Crouch et al. (1983) *J. Mol. Appl. Genet.*, *2*; 273-283. Briefly, at 0-4°C, tissue was ground to powder in liquid nitrogen and the powder was resuspended in homogenization buffer (0.1 M Tris-HCL (pH 9.0), 0.1 M NaCL, 1 mM EDTA (pH 8.0), 0.5% SDS) at 20 mL buffer per gram of tissue (v/w). This was done at 0-4°C. One-half volume of hot phenol, which had been previously equilibrated with homogenization buffer was then added and the mixture was homogenized using a Brinkman polytron at high speed for one minute. One-half volume of SEVAG was then added and the mixture was homogenized as before. The aqueous phase was separated by centrifugation at 8000 x g for 10 minutes and removed. The phenol/SEVAG extraction was repeated and the aqueous phase was removed. Nucleic acids were precipitated in 0.2 M potassium acetate (pH 6.0) and 2.5 volumes ETOH overnight at -20°C. The homogenate was ethanol precipitated once more followed by lithium chloride and potassium acetate precipitations before a final ethanol precipitation. The RNA was stored as an ethanol precipitate at -90°C until use. Before using the RNA in enzymatic reactions, the precipitate was washed in cold 70% ethanol followed by a cold 95% ethanol wash and resuspended in TE buffer.

Differential display analysis

Differential display analysis was routinely carried out using 1 mg total RNA per sample as starting material. cDNA synthesis was carried out as described previously (Liang et al., 1992; Liang et al. (1993) *Nucl. Acids Res., 21*; 3269-3275). The first-strand cDNA template was synthesized using reagents from the GIBCO-BRL cDNA synthesis kit (Cat. #18267-013). Total RNA was incubated in 22.5 ml containing 5 ml 5X reaction buffer (250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM $MgCl_2$), 2.5 ml 200 mM dNTPs, and 2.5 ml 25 mM $T_{11}VN$ primer (where V=dATP, dCTP, or dGTP and N=dATP, dCTP, dGTP or dTTP) for 3 minutes at 65°C, then allowed to cool for 3 minutes at room temperature. This was repeated twice more. Dithiothreitol was added to a final concentration of 5 mM and 250 Units of MMLV reverse transcriptase were added and the cDNA synthesis reaction was carried out at 37°C for 1 hour. The reaction was terminated by heating to 95°C for 5 minutes. This represents the cDNA template used for the differential display PCR reaction and was stored at -20°C until use.

The PCR reaction also followed earlier protocols (Liang et al., 1992; Liang et al., 1993), but the reaction components varied depending on the radioactive probe used to identify the reaction products. When ^{32}P-dATP was used, the final dNTP concentration was 2 mM. When a ^{32}P-labeled primer was used, the final dNTP concentration was 200 mM except where it is otherwise indicated. The $T_{11}VN$ primer or the arbitrary 10-mer were end-labeled as follows: 3.125 nmole primer was incubated with 125 pmole ^{32}P-g-ATP in a kinase reaction described in Ausubel et al. (1994) *Current Protocols in Molecular Biology*, New York: John Wiley and Sons. The labeled primer was precipitated with one half volume of 7.5M ammonium acetate and 2.5 volumes 100% ethanol using 50 mg glycogen as carrier at -85°C for 1 hour. The pellet was washed briefly with 95% ethanol, dried and resuspended in 50 ml TE buffer.

The PCR reactions were set up as follows: 2 ml cDNA template (representing 40 ng of the original total RNA) and 2.5 mM $T_{11}VN$ primer (the same primer used to prime first strand cDNA synthesis) were added to a reaction mix containing 0.5 mM arbitrary 10-mer, 50mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 4.8 mM $MgCl_2$, either 2 mM or 200 mM of each dNTP, and 5 Units of *Taq* polymerase (Promega, Madison, WI) in a final volume of 25 ml. The reaction

mix was overlayed with mineral oil and heated to 85°C for 5 minutes followed by a thermocycle program of 95°C for 30 seconds, either 42°C or 35°C for 1 minute, 72°C for 30 seconds and cycled 40 times. This was followed by a 5 minute extension period at 72°C. The reaction products were resolved by adding 3 ml sequencing reaction stop buffer (Epicenter Technologies) to 6 ml of reaction mix and resolved on a 6% sequencing gel at 50 mAmps. The gel was dried and autoradiographed.

Differential display bands were excised as described previously (Liang et al., 1992). The gel slice was placed in a dialysis bag containing 300 ml lX TBE buffer and electroeluted as described in Ausubel et al. (1994). The eluent was collected, and the DNA was precipitated as described above. The pellet was washed briefly in 95% ethanol, dried and resuspended in 10 ml TE buffer. DNA representing the differential display band was regenerated using 4 ml of the isolated DNA in a reaction similar to the differential display PCR reaction except that 2.5 mM unlabeled $T_{11}VN$ primer used previously. A 1 ml aliquot was resolved in a 1% agarose gel which was photographed, dried and autoradiographed. A successful regeneration was characterized by the appropriately sized band which demonstrated radioactivity above background. The remaining reaction products were resolved on a 1% agarose gel and the DNA representing the regenerated band was excised and isolated from the agarose by centrifugation through a 45 mM microspin filter as described by the manufacturer (Millipore). The DNA was precipitated and dissolved in a final volume of 20 ml TE. This DNA represents the template used to generate the differential display probes.

Synthesis of differential display probes

The regenerated differential display band was used as template to generate the differential display probe. The probe was synthesized in the following PCR reaction: 2 ml of regenerated DNA was combined in a reaction mix containing 2.5 mM $T_{11}VN$ primer; 0.5 mM arbitrary 10-mer; 50 mM KCl; 10 mM Tris-HCl (pH 9.0 at 26°C); 0.1% Triton X-100; 4.8 mM $MgCl_2$; 207 mM dCTP, dGTP, and dTTP; 7 mM dATP; 50 mCi ^{32}P-dATP (3000 Ci/mmol), and 5 Units of *Taq* polymerase (Promega, Madison, WI), in a final volume of 30 ml. The reaction mixture was overlayed with mineral oil and subjected to a thermocycling program identical to that described for the differential display PCR reaction. Unincorporated reaction products were removed by

centrifugation through a G-50 spin column (Boehringer Mannheim, Indianapolis, IN). The ^{32}P-incorporation was measured by scintillation counting and the probe was used at a final concentration of at least $1x10^6$ cpm/ml.

Plaque hybridization

An *Arabidopsis thaliana* var. Landsberg erecta cDNA library representing immature seeds was constructed following the method of *Nuccio et al.* (1996). The library was plated on XL1-Blue MRF' cells at a density of 50,000 PFU per plate (150 mM) containing LB media. Plaques were transferred to nitrocellulose membranes as recommended by the manufacturer and hybridized by standard methods (Ausubel et al., 1994). After 4 hours prehybridization in hybridization II buffer (1% crystalline BSA, 1 mM EDTA, 0.5 M $NaHPO_4$, pH 7.2, 7% SDS) at 65°C, the differential display probe, which had been boiled in 50% formamide for 3 minutes, was added to the same hybridization solution. Hybridization was continued up to 24 hours at 65°C. The filters were washed twice in 0.5% crystalline BSA, 1 mM EDTA, 40 mM $NaHPO_4$, pH 7.2, 5% SDS for 5 minutes each at room temperature, and then three times in 1 mM EDTA, 40 mM $NaHPO_4$, pH 7.2, 1% SDS for 10 minutes each at 65°C. Autoradiographs were exposed for 1 day at -85°C.

RNA gel blot analysis

10 mg of total RNA from flower, leaf, root, immature seed, and silique without seed were resuspended in 10 ml loading buffer (48% formamide, 1X MOPS buffer 0.02 M 3-[N-morpholino] propane sulfonic acid, 1mM EDTA, 5 mM sodium acetate at pH 6.0), 17% formalin, 0.7 mg/ml ethidium bromide, 5.3% glycerol, 5.3% saturated bromophenol blue) and resolved on a 1.2% agarose gel containing 7% formaldehyde in 1X MOPS buffer. RNA was transferred to a nylon filter (Micron Separations Incorporated) in 10X SSC. Blots were hybridized with probes prepared from gel purified cDNA inserts in 50% deionized formamide, 5X SSPE, 1X Dendhardt's solution, 0.1% SDS, and 100 mg denatured salmon sperm DNA at 42°C for 24 hours. Radioactive probes were prepared from cDNA templates by the random primer method (Feinberg et al. (1983) *Alan. Biochem., 132*; 6-13) and each had a specific activity greater than $1x10^9$cpm/mg. Filters were washed first in 0.6 M NaCl, 0.08 M Tris-HCl, 4 mM EDTA, 12.5 mM phosphate buffer, pH 6.8 and 0.2% SDS at

60°C for 15 minutes, followed by 0.3 M NaCl, 0.04 M Tris-HCL, 2 mM EDTA, 12.5 mM phoisphate buffer, pH 6.8, and 0.2% SDS at 60°C for 15 minutes, and then 0.15 M NaCl 0.02 M Tris-HCl, 1 mM EDTA, 12.5 mM phosphate buffer, pH 6.8 and 0.2% SDS at 60°C for 10 minutes. The filters were wrapped in Saran Wrap and autoradiographed.

Sequence analysis

Mini-prep plasmid DNA was used as templates in cycle sequencing reactions with the SequiTherm cycle sequencing kit (Epicenter Technologies, Madison, WI). Sequence analysis was done locally with GCG (Devereux et al. (1984) *Nucl. Acids Res., 12*; 387-395) on a DEC Micro VAXII; database searches were done remotely through NCBI using the BLAST algorithm (Altschul et al. (1990) *J. Mol. Biol., 215*; 403-410). cDNAs representing previously characterized *Arabidopsis* genes were discarded

EXAMPLE 2 CHARACTERIZATION OF *ATS1* AND *ATS3* BY DEVELOPMENTAL RNA GEL BLOT ANALYSIS

RNA Gel Blot Analysis

A total of ten groups of putative seed specific cDNAs were identified in the cDNA library screen (Table 1.).

In Table 1, (a) the sequences of the arbitrary 10-mers used for the differential display experiment are: A10 (5'-gtgatcgcag-3'), A12 (5'-tcgccgatag-3'), ca/b(5'-ctagcttggt-3'), (b) means the number of cDNAs plaque purified from the *Arabidopsis* immature seed cDNA library with the differential display probe. In each screen a total of 12 individual hybridizing plaques were targeted. (c) means the number of individual genes represented by the pool of plaque purified cDNAs, (d) represents the unique genes in the cDNA pool (they are not represented in GeneBank), and (e) means the cDNA probe recognizes a seed-specific mRNA.

Only three of these putative seed specific cDNAs were verified to be seed-specific by RNA gel blot analysis. The differential display gels identifying AtS1 and AtS3 are depicted in Figures 1A and 1B, respectively.

TABLE 1 ANALYSIS OF PUTATIVE SEED SPECIFIC DIFFERENTIAL DISPLAY PRODUCTS

Product	10-mer used[a]	Size (bp)	cDNAs purified[b]	Genes represented[c]	Number of unique sequences[d]	RNA gel blot confirmation[e]	Designation	Comments
ddp1	A12	450	6	3	1	no		
ddp2	A12	370	5	3	2	no		
ddp3	A12	300	10	5	2	no		
ddp4	A10	710	12	6	2	no		
ddp5	A10	500	12	3	1	yes	AtS1	This cDNA is represented 6 times
ddp6	A10	150	8	5	2	no		
ddp7	ca/b	475	7	4	2	yes	AtS2	The cDNA was chimeric
ddp8	ca/b	450	12	4	1	yes	AtS3	This cDNA is represented 6 times
ddp9	ca/b	300	12	9	2	no		
ddp10	ca/b	250	10	9	5	no		

The cDNA designated AtS2 is a confirmed seed-specific cDNA, and the initial sequence analysis indicated that it was novel. Further sequencing, however, revealed that it was chimeric and contained a fragment of 12S seed storage protein sequence. Subsequent RNA gel blot analysis indicated that the 12S component of this clone was responsible for the seed-specific signal. Thus, it was discarded.

The cDNAs isolated by differential display analysis in Example 1 were then subjected to expression analysis by RNA gel blot hybridization. This step was performed in order to confirm results from the differential display analysis.

Arabidopsis thaliana (Landsberg) growth conditions and tissue preparation were as described in Example 1. RNA was also prepared as described in Example 1. Tissue representing globular-heart (1-3 day post flowering), heart to torpedo (3-5 day post flowering), torpedo to early cotyledon (5-7 day post flowering), early cotyledon to late cotyledon (7-13 day post flowering) stage siliques was collected and stored at -90°C. Dry seeds, floral and leaf tissue were also collected. Ten micrograms of total RNA were resuspended in 10 ml loading buffer (48% formamide, 1X MOPS buffer 0.02 M 3-[N-morpholino] propane sulfonic acid, 1mM EDTA, 5mM sodium acetate at pH 6.0), 17% formalin, 0.7 mg/ml ethidium bromide, 5.3% glycerol, 5.3% saturated bromophenol blue) and resolved on a 1.2% agarose gel containing 7% formaldehyde in 1X MOPS buffer. RNA was transferred to a nylon filter (Micron Separations Incorporated, Westboro MA) in 10X SSC. Blots were hybridized with probes prepared from gel purified cDNA inserts in 50% deionized formamide, 5X SSPE, 1X Denhardt's solution, 0.1% SDS, and 100 mg denatured salmon sperm DNA at 42°C for 24 hours.

Radioactive probes were prepared from cDNA templates representing both the AtS1 and AtS3 genes, a tubulin gene (Marks et al. (1987) *Plant Mol. Biol., 10*; 91-104), the 12S cruciferin gene and the 2S albumin gene (Guerche et al. (1990) *Plant Cell., 2*; 469-478; Pang et al, 1988) by the random priming method (Feinberg et al., 1983) and each had a specific activity of greater than $1x10^9$ cpm/ug. Filters were washed first in 0.6M NaCl, 0.08 M Tris-HCl, 4 mM EDTA, 12.5 mM phosphate buffer, pH 6.8, and 0.2% SDS at 60°C for 15 minutes, and then 0.3 M NaCl, 0.04 M Tris-HCl, 2 mM EDTA, 12.5 mM phosphate buffer, pH 6.8, and 0.2% SDS at 60°C for 15

minutes, followed by 0.15 M NaCl, 0.02 M Tris-HCl, 1 mM EDTA, 12.5 mM phosphate buffer, pH 6.8, and 0,2% SDS at 60°C for 10 minutes. Hybridization signals were recorded with a Fujix BAS 2000 phosphoimager. The data were analyzed using MacBAS (ver. 2.1) software. The hybridization signal was quantitated and adjusted for probe specific activity and length. The hybridization signal for each sample was also adjusted for loading by virtue of hybridization to a tubulin cDNA probe (Marks et al., 1987).In this manner, both the quantitative and temporal accumulation of the AtS1 and AtS3 genes were determined and compared to that of well characterized seed-specific genes.

TABLE 2 DEVELOPMENT EXPRESSION OF FOUR SEED SPECIFIC *ARABIDOPSIS GENES*

	Hybridization*					
Probe	leaf	g-h	h-t	t-ec	ec-lc	dry
12S cruciferin	1	14	123	748	1510	454
2S albumin	0	2	8	172	355	73
AtS1	0	1	2	11	36	9
Ats3	0	0	1	19	54	1

* The data represents the hybridization signal and is presented in arbritrary units which have been normalized for laoding, probe-specific activity and probe length.

EXAMPLE 3 CHARACTERIZATION OF ATS1 AND ATS3 BY *IN SITU* HYBRIDIZATION

In situ hybridization analysis was used to establish the spatial accumulation of mRNA for each of the AtS1 and AtS3 genes. This approach utilized a digoxygenin-labeled RNA probe which was detected with an antibody conjugated to alkaline phosphatase. It was determined that this was the most reliable method to detect gene expression at the cellular level in developing *Arabidopsis* seeds.

Tissue representing developing *Arabidopsis* seeds and germinating seedlings was collected and fixed in a solution containing 4% formaldehyde and 0.5% glutaraldehyde in 100 mM phosphate buffer (pH 7.0) at 0°C overnight. The tissue was

dehydrated in 10%, 30%, 50%, 70%, 85%, 95%, and 100% ethanol three times for thirty minutes at room temperature for each step. The solvent was gradually changed to xylenes in the following series 25%, 50%, 75% and 100% three times at room temperature. An equal amount of Paraplast (Sigma, St. Louis, Mo) was added to the xylenes and incubated overnight at room temperature. The mixture was then placed at 42° for 6 hours. It was decanted off, replaced with 100% molten paraplast and placed at 60°C. The paraplast was replaced four times at four hour intervals to remove all the xylenes. The paraplast embedded tissue was then poured into molds and cooled to room temperature. The embedded tissue was kept in a desiccated container at room temperature until sectioning.

Tissue was sectioned into 8 mm ribbons with a Lipshaw Model 50A microtome. The ribbons were overlayed on DEPC treated H_2O on poly-L lysine coated microscope slides on a 45°C slide warmer. The water evaporated overnight, fixing the sections to the slides. The slides were stored at room temperature.

The digoxygenin labeled riboprobes were prepared with the Genius™ 4 nonradioactive RNA *in vitro* transcription kit (Boehringer Mannheim, Indianapolis, IN). The cDNAs encoding the AtS1 and AtS3 genes were cloned into pBluescript (SK) as *EcoRI/XhoI* fragments. The template for antisense riboprobes was generated by an *EcoRI* digest, gel purified and quantitated. To generate the template for sense strand riboprobes, each cDNA was excised from pBluescript (SK) as *EcoRI/XhoI* fragments and cloned into pBluescript (KS) as the same. The template for the sense-strand riboprobe was constructed in the same method as the template for the antisense probe. Each riboprobe was synthesized in a reaction containing 2 mg linearized DNA template, 2 ml 10X T7 RNA polymerase buffer, 2 ml 10X NTPs containing digoxygenin-UTP, 1 ml RNAse inhibitor and 2 ml T7RNA polymerase (5U) in a 20 ml reaction. The reaction was incubated at 37°C for 2 hours. The DNA template was digested with 5 Units of RNAse-free DNAse (Boehringer Mannheim, Indianapolis, IN) for 5 minutes at 37°C. The digoxygenin-labeled riboprobe was then purified over a G-50 spin column (Boehringer Mannheim, Indianapolis, IN) and ethanol precipitated.

Each riboprobe was sheared into strands averaging 100-200 bases by alkali treatment. RNA pellets were dissolved in 22 ml DEPC treated H_2O. Only 20 ml

of the redissolved riboprobe was sheared with the addition of 20 ml 120 mM Na_2CO_3, 80 mM $NaHCO_3$ and incubating at 65°C for 35 minutes. The reaction was terminated with the addition of 40 ml sodium acetate and the riboprobe was ethanol precipitated. The remaining riboprobe was reserved for gel analysis. Each riboprobe was resuspended in DEPC H_2O, quantitated and analyzed by gel electrophoresis. The riboprobes were kept at -90°C until use.

The slides were prepared for hybridization first by removing the paraplast by immersion in 100% xylenes twice for 10 minutes each. The slides were transferred to 1:1 xylenes:ethanol for five minutes followed by 100% ethanol for two changes of 10 minutes each to remove the xylenes. The slides were then rehydrated through a series (ddH_2O:ethanol) of 5%, 15%, 30%, 50%, 70%, 85% and 95% ddH_2O for five minutes each step. The slides were finally transferred to PBS (50 mM phosphate buffer(pH 7.0), 130 mM NaCl in DEPC H_2O for two 5 minute incubations at room temperature. The slides were then incubated in 50 mM phosphate buffer (pH 7.0) containing 100 mg/ml proteinase K for 15 minutes at 37°C. The digests were stopped by two washes in PBS for five minutes each.

The tissue was then acetylated by incubation in fresh 1% triethanolamine (pH8.0), 0.5% acetic anhydride for 10 minutes at room temperature. The reaction was terminated by two washes in PBS for 5 minutes each. This was followed by a quick dehydration series in 5%, 15%, 30%, 50%, 70%, 85%, 95%, and two times 100% ethanol. The slides were air dried and kept at room temperature until the hybridization.

Each riboprobe was diluted to 300 ng/ml in hybridization solution containing 50% deionized formamide, 300 mM NaCl, 10 mM Tris-HCl(pH 7.5), 5 mM EDTA (pH 8.0), 1X Dendhart's solution, 10% dextran sulfate, 1 mg/ml yeast tRNA and 500 mg/ml poly-A RNA. The hybridization mixture was overlayed on each dried slide (250 ml per slide), covered with a coverslip, and incubated overnight in a moist container at 50°C.

The unhybridized probe was removed by washing the slides in 2X SSC/50% deionized formamide 4 times for 30 minutes each at 50°C. The slides were then washed in NTE buffer (500 mM NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA (pH 8.0)) twice at 37°C for 10 minutes each. The slides were then treated with 20U/ml

RNAse A plus 20 mg/ml RNAse T1 in NTE buffer for 30 minutes at 37°C. The RNAse cocktail was removed by 4 washes in NTE buffer at 37°C for 30 minutes each. The slides were washed in 2X SSC/50% deionized formamide at 50°C for 30 minutes and then washed in PBS at room temperature twice for 10 minutes each.

The slides were then incubated in Buffer I (100 mM Tris-HCl (pH 7.5), 150 mM NaCl) for 30 minutes at room temperature. The slides were blocked in Buffer I containing 1% BSA or gelatin at room temperature for 30 minutes. An anti-digoxigenin Fab fragment conjugated with alkaline phosphatase (Boehringer Mannheim) was diluted 1:2500 in Buffer I containing 1% BSA or gelatin and 500 ml was added to each slide. The slides were covered with cover slips and incubated at room temperature for one hour. The unhybridized antibody was removed with 4 washes in Buffer I at room temperature for 15 minutes each. The slides were rinsed in Buffer III (100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 50 mM $MgCl_2$) for two minutes at room temperature and incubated in color solution to detect hybridization. The color solution contained 337.5 mg/ml NBT (nitroblue tetrazolium) and 175 mg/ml X-phosphate (5-bromo-4-chloro-3indolyl phosphate) in Buffer III. The color reaction was carried out for 2 hours to 3 days, depending on the experiment.

The color reactions were stopped by washing slides in deionized H_2O. The slides were dehydrated quickly in 30%, 50%, 70%, 85%, 95% and 100% ethanol and air dried. The samples were preserved in several drops of either Euparal (BioQuip Products, Inc., Gardena, CA) or Permount (Fisher, Fair Lawn, NJ) and a cover glass was mounted. The mounted samples were dried for several days at room temperature. Micrographs of individual sections were taken with a Zeiss Axiophot microscope using DIC optics.

The *in situ* hybridization data is presented in Figures 5A through 5F and Figures 6A through 6F. The mRNA for both genes is first detected at the late torpedo stage. Expression above background was not detected in earlier embryos. As indicated in Figures 5C through 5F, the AtSI gene is expressed throughout the maturing embryo. Expression is initially detected in the cortical parenchyma and gradually spreads throughout the embryo as it matures. Figures 5E and 5F indicate that expression levels are significantly enhanced in both the protoderm and vascular initials in the cotyledon

stage embryo. This pattern is clearly seen in the cross sections (Figures 5D and 5E), but was not detected in longitudinal sections (Figure 5F). In developing *Arabidopsis* embryos, a similar pattern was reported for the GEA1 gene (Gaubier et al. (1993) *Mol. Gen. Genet., 238*; 409-418), indicating that the expression profile may not be unique to the AtSl gene.

The *in situ* hybridization data for the AtS3 gene is resented in Figures 6A through 6F. The AtS3 gene is expressed in a pattern that closely resembles both the 2S and 12S genes with the earliest signals detected in the cortical parenchyma at the torpedo stage (Guerche et al., 1990; Pang et al., 1988). There is no expression detected in the procambium or the root and shoot apical meristems. This likely indicates that the AtS3 gene product is either a minor seed storage protein or is involved in the stable accumulation of seed storage proteins.

These data indicate that, while both genes are expressed in a similar temporal pattern, their spatial accumulation in the developing embryo is distinct. Furthermore, the expression of both genes is restricted to the developing embryo. No expression was detected in the embryo sac, endosperm or the germinating seedling, even after several days exposure to the calorimetric agent. Also, no signal was detected with sense strand riboprobes. This indicates that both AtS1 and AtS3 are involved in developmental processes unique to the maturing embryo. Due to their unique spatial expression however, each gene may be involved in distinct regulatory programs.

EXAMPLE 4 AtS1 AND AtS3 GENE ORGANIZATION

Genomic clone isolation

Genomic DNA was prepared from *Arabidopsis* (cv. Landsberg) according to Taylor et al. (1993) *Methods in Plant Molecular Biology and Plant Biotechnology,* Boca Raton, FL: CRC Press; 37-47. The DNA was partially digested with *MboI* and overlayed on a sucrose gradient for size selection (Ausubel et al., 1994). Fractions containing DNA fragments ranging from 15-25 kb were combined and precipitated. The DNA was dissolved in TE buffer, quantitated and ligated to lambda pGEM-11 *XhoI* half-site arms according to manufactures' instructions (Promega, Madison, WI). The DNA was packaged using Gigapack Gold packaging extracts

(Stratagene, La Jolla, CA) and plated on KW251 cells. Characterization of this library revealed a 1% background and an average insert size of 20 kb. The library contained approximately $1.5x10^6$ plaque forming units and was amplified and stored in SM buffer containing $CHCl_3$ at 4°C.

Approximately 25,000 pfu of this library was plated on KW251 cells. Plaques were transferred to nitrocellulose membranes as recommended by the manufacturer and hybridized by standard methods (Ausubel et al., 1994). After 4 hours of prehybridization in hybridization II buffer (1% crystalline BSA, 1 mM EDTA, 0.5 M $NAHPO_4$, pH 7.2 7% SDS) at 65°C, the random-primed DNA generated from either an AtS1 or AtS3 cDNA template, which had been boiled in 50% formamide for 3 minutes, was added to the same hybridization solution. Hybridization was continued up to 24 hours at 65°C. The filters were washed twice in 0.5% crystalline BSA, 1 mM EDTA, 40 mM $NaHPO_4$, pH 7.2, 5% SDS for 5 minutes each at room temperature, and then three times in 1 mM EDTA, 40 mM $NaHPO_4$, pH 7.2, 1% SDS for 10 minutes each at 65°C. Autoradiographs were exposed for 1 day at -95°C. Several phage were plaque purified with the AtS1 cDNA probe while only one clone was plaque purified with the AtS3 probe. Phage DNA was prepared using the liquid lysate protocol (Ausubel et al., 1994) and aliquots were separately digested with *BamHI*, *EcoRI*, *HindIII*, *SacI*, and *XbaI*. The AtS1 probe identified a 5.5 kb *SacI* fragment and the AtS3 probe identified an 8.0 kb *XbaI* fragment. These were subcloned into pBluescript (Stratagene, La Jolla, CA) and sequenced.

Southern analysis

Arabidopsis genomic DNA was isolated from whole plants according to the CTAB (hexadecyltrimethylammonium bromide plant genomic DNA preparation protocol (Taylor et al., 1993). Genomic DNA (10 mg) was digested in the presence of excess enzyme activity at 37°C overnight and then resolved on a 0.7% agarose gel. Separate digestions using *BamHI*, *EcoRI*, *HindIII*, *SacI* and *XbaI* were performed on the genomic DNA. DNA was transferred by blotting to Hybond-N^{+TM} membrane (Amersham) with 0.1 N NaOH. Southern hybridizations were performed essentially as described for the genomic clone isolation. After 4 hours prehybridization in hybridization II buffer (1% crystalline BSA, 1 mM EDTA, 0.5 M $NaHPO_4$, pH 7.2, 7%

SDS) at the hybridization temperature, the random-primed DNA probe generated from either an AtS1 or AtS3 cDNA template, which had been boiled in 50% formamide for 3 minutes, was added to the same hybridization solution. Hybridization was continued up to 24 hours. The filters were washed twice in 0.5% crystalline bSA, 1 mM EDTA, 40 mM $NaHPO_4$, pH 7.2, 5% SDS for 5 minutes each at room temperature, and then, stringently, three times in 1 mM EDTA, 40 mM $NaHPO_4$, pH 7.2, 1% SDS for 10 minutes each at high temperature. The high stringency hybridizations were performed at 68°C and the stringent washing steps were done at the same temperature. The low stringency hybridizations were done at 50°C and the stringent washing steps were done at 60°C.

High stringency southern hybridization analysis of *Arabidopsis* genomic DNA indicated that both genes were present as single copies in the diploid genome (Figure 7A). Southern hybridization analysis under low stringency revealed that the AtS1 probe hybridizes to two or three additional bands depending on the digest. Clone blot analysis of these phage indicate that each contains a hybridizing fragment identical to a band uncovered by the low stringency genomic southern blot, Figure 7B. The clones which contained a hybridizing fragment corresponding to a band in the high stringency genomic DNA analysis, indicated by the arrows in Figure 7A were identified. This corresponds to a 5.5 kb *SacI* fragment for AtS1 and an 8.0 *XbaI* fragment for AtS3. The DNA representing these bands was subcloned into pBluescript and completely sequenced.

DNA sequencing and sequence analysis

Mini-prep plasmid DNA was used as templates in cycle sequencing reactions with the SequiTherm cycle sequencing kit (Epicenter Technologies, Madison, WI) or the ABI PRISM™ dye terminator cycle sequencing kit (Perkin Elmer, Foster City, CA). Sequence analysis was done locally with GCG (Devereux et al., 1984) on a DEC MicroVAXII; database searches were done remotely through NCBI using the BLAST algorithm (Altschul et al. (1990) *J. Mol. Biol., 215*; 403-410).

Genomic and cDNA sequence data for each gene was aligned using Geneworks, Version 2.3 software (Intelligenetics, Mountain View, CA). Introns were initially located with a DNA dot matrix algorithm. Inspection of these regions found

them to be flanked by consensus GU...AG sequence. The downstream genes identified on each genomic clone were found using BLAST and BLASTX data search algorithms (Altschul et al., 1990). The longest open reading frame found in each cDNA was considered to be the coding sequence and the codon for that methionine residue was labeled +1. The coding sequence was translated from that residue and hydrophobicity plots were generated using the Kyte-Doolittle algorithm.

Each genomic clone contained a complete target gene, including at least 1.3 kB of 5'-untranslated sequence. Alignment of the longest cDNA clone with each genomic clone revealed that the putative coding sequence is interrupted with introns with the consensus GU...AG borders. The data presented in Figures 8 and 11A indicate that AtS1 contains five introns and six exons, while Figures 9 and 11B indicate that AtS3 contains two introns and three exons. Alignment of several individual cDNAs with each genomic clone revealed that each transcript is terminated at a different position along a 120-300 base track (Figure 4). Thus the AtS1 mRNA has at least a 185-300 base 3'-untranslated region, and the AtS3 mRNA has at least a 127-179 base 3'-untranslated region. The poly-adenylation sites are indicated by an asterisk in Figure 8 and Figure 9, respectively. No consensus poly-adenylation signal sequence was noted in the 3'-untranslated region of either cDNA, indicating that there is not a consensus poly-adenylation site in either gene.

The AtS1 and AtS3 genomic regions

Sequence analysis of the genomic regions downstream of both the AtS1 and AtS3 genes reveal that additional transcribed genes lie in close proximity. Figures 11A and 11B are diagrams detailing the known transcribed regions in the AtS1 and AtS3 genomic clones. As indicated in Figure 11A, the gene encoding the *Arabidopsis* protein phosphotaseX, PPX2 (Perez-Callejon et al. (1993) *Plant Mol. Biol., 23*; 1177-1185), lies directly downstream of, and in tandem with, the AtS1 gene. The translation start codon is 1630 base pairs 3' of the AtS1 translation stop codon site. The sequence reported for this gene is identical to the sequence found in the AtS1 genomic clone. The PPX-2 gene is not expressed in the same pattern as that of the AtS1 gene. For example, PPX-2 gene expression was detected at relatively low levels in all tissues examined (Perez-Callejon et al., 1993). It is not known if any previously identified genes lie

upstream of the AtS1 gene.

Figure 11B indicates that the AtS3 genomic clone contains at least one additional transcribed gene. Two anonymous, overlapping cDNAs (GeneBank accession numbers Z30724 and T45484) align with the genomic DNA. These cDNAs identify a region spanning bases 4342-4845 in the AtS3 genomic clone, which is 1916-2419 bases downstream from the AtS3 translation stop condon. This gene is transcribed off the DNA strand opposite the AtS3 gene. Both of these sequences were identified in independent expressed sequence tag (est) projects. Structural analysis of these cDNAs reveal nothing regarding this gene's possible function in the plant.

EXAMPLE 5 FURTHER ANALYSIS OF GENOMIC AtS1 and AtS2 CLONES

Mapping of Transcription sites by RNAse Protection Analysis

The transcription start sites for both AtS1 and AtS3 were mapped by RNAse protection assay.

First, the riboprobes used to map the transcription start sites for AtS1 and AtS3 were constructed. A region encompassing the 5'-region of each cDNA was amplified in a *Pfu* polymerase reaction, gel purified and cloned into EcoRV digested pBluescript (SK-). The primers used to generate the AtS1 template were 5'-ttattattacctc-3' (primer T5RP)(SEQ ID NO:29) and 5'-gaagtctatcatcc-3' (primer T3RP) (SEQ ID NO:30) which yield a 189 bp fragment. The primers used to generate the AtS3 template were 5'-cactcacgagtgcctc-3' (primer 8g.5P)(SEQ ID NO:31) and 5'-acaagaagaacctgg-3' (primer 8g.3P)(SEQ ID NO:32) which yield a 166 bp fragment. Both fragments were oriented so that the antisense riboprobe was transcribed from the T7 promoter. Each clone was linearized with an EcoRI digest and gel purified. Approximately 2 mg of linearized template was used in an *in vitro* transcription reaction to produce high specific activity probe according to the manufacturer's instructions (Stratagene, La Jolla, CA). The probe was gel purified on a non-denaturing acrylamide gel. Bands representing full-length transcript were excised and the probe was eluted into TE buffer overnight at 37°C. Incorporation of radioactive label was measured and the probes were used immediately.

Total RNA was prepared from dry *Arabidopsis* seed. The RNAse

protection experiment was performed using the Direct Protect kit (Ambion, Austin, TX). The manufacturer's instructions were used with the exception of the following modifications. First, it was determined that a better signal was achieved when total RNA prepared from dry seeds was substituted for tissue. Second, approximately 250,000 cpm of probe was used with each sample. Total RNA amounting to 0, 2, 5, 10, and 20 mg were combined with probe and lysis buffer in a total volume of 50ml and incubated overnight at 37°C. The reactions were completed according to manufacturers instructions and the protected fragments were resolved on a 6% sequencing gel along with a sequencing reaction primed by the 3'-terminal primer (AtS1-T3RP and AtS3-8g.3P) used to generate each riboprobe template. Protected fragments were identified as bands demonstrating increasing intensity with increasing total RNA template concentration. The size of the protected fragments was determined by comparing the size of co-migrating DNA ladder generated by the sequencing reaction (Calzone et al. (1987) *Methods in Enzymology, 152*; 611-632). Since a protected fragment did not co-migrate with the undigested probe, it was assumed that the transcription start site for each gene was contained within the boundaries of the riboprobe template.

Experimental results are detailed in Figures 10A (AtS1 gene) and 10B (AtS3 gene). Protected fragments, indicated by the arrows, were identified as titrated bands on a sequencing gel. Bands which did not titrate were ignored. Bands equal to or greater than each probe's length were not detected, indicating that no other transcription start site occurs upstream of the sequence analyzed. This data reveals two transcription start sites in the AtS1 gene (Figure 10A) and four in the AtS3 gene (Figure 10B). These sites are indicated by a double underline in Figures 8 and 9, respectively. The signal strength indicates that the AtS1 gene is preferentially transcribed from the site that is more proximal to the translation start site, while the AtS3 gene does not appear to have a preferential site. A putative TFIID binding site and CAT box were also identified upstream of each transcription start site (Figures 8 and 9).

EXAMPLE 6 ESTABLISHMENT OF SEED SPECIFICITY FOR THE GENE PRODUCTS OF AtS1 and AtS3

Antisera production

DNA representing the putative coding sequence for both the AtS1 and AtS3 genes was subcloned into the pET expression vector pET-30a(+) (Novagen, Madison, WI). The coding sequence for AtS1 was excised from the cDNA ddp5(8) as an *EcoRI/XhoI* fragment and ligated directly into the expression vector. To generate an in frame fusion with the AtS3 coding sequence, two primers, 5'-accgaattca tggcattcga cctcagcatc-3' (AtS3-5' del)(SEQ ID NO:33) and 5'-cgtgagctct cactaatttc caagccttga agc-3' (AtS3-3' del)(SEQ ID NO:34), were used in a *Pfu* polymerase reaction to amplify the coding sequence. The *Pfu* product was digested with EcoRI/SacI, gel purified and ligated into the pET-30a(+) expression vector. The integrity of each coding sequence was verified by sequence analysis.

Fusion proteins for both AtS1 and AtS3 were generated and purified by affinity chromatography on a nickel column according to manufacturers instructions (Novagen, Madison, WI). The integrity of each purified fusion protein was verified by SDS/PAGE and western analysis. Each protein was combined with RIBI adjuvant and injected subcutaneously into rabbits to raise polyclonal antibodies against the AtS1 and AtS3 gene products. Each antibody was then used in western and light level immunolocalization analysis to establish the seed specificity of both gene products.

Western analysis

Total protein was extracted from fresh plant tissue by homogenizing fresh tissue in protein extraction buffer (50 mM $NaPO_4$ (pH 7.0), 150 mM NaCl, 10 mM EDTA, 10 mM 2-mercaptoethanol, 0.1% sodium sarcrosyl, 0.1% Triton X-100, 4% sodium dodecyl sulfate, 2 M urea) at 4°C. Insoluble material was separated by centrifugation at 13,000xg for 10 minutes at 4°C. The supernatant was removed and total protein was measured by the method of Bradford (1976) *Anal. Biochem., 72*; 248-254. Total protein was resolved on a 12.5% denaturing polyacrylamide gel and electroblotted onto nitrocellulose (Ausubel et al., 1994). The filter was incubated in blocking solution (10 mM Tris-HCl (pH7.5), 150 mM NaCl, 1% BSA, 0.2% NP-40) for 30 minutes at room temperature. Primary antiserum was diluted in blocking solution as

indicated and incubated overnight at room temperature. The filter was washed four times in washing solution (10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% SDS, 0.2% NP-40, 0.25% sodium deoxycholate) for 15 minutes each at room temperature. This was followed by a rinse in 10 mM Tris-HCl (pH 7.5), 150 mM NaCl to remove detergent for 10 minutes at room temperature. The filter was then incubated in blocking solution containing 1:5000 goat anti-rabbit FAB fragment conjugated to alkaline phosphatase for 1 hour at room temperature. The filter was washed as described for the primary antibody. The hybridization was detected through an alkaline phosphatase reaction.

As the Western blot indicates in Figures 12A and 12B, each antibody specifically reacts with a band in immature seed tissue. This data indicates that the open reading frame for both AtS1 and AtS3 has been correctly interpreted. The band recognized by the AtS1 antibody has a molecular weight of 33 kD, somewhat larger than the predicted 28,020 Dalton from the cDNA sequence. This discrepancy might indicate that the native protein is either covalently modified to produce the mature protein or that it migrates at a slower than predicted rate in the gel. The AtS3 antibody specifically recognizes a 30kD band. This is also somewhat larger than the predicted molecular weight of 23,042 Daltons. In the case of both AtS1 and AtS2, the antibodies do recognize seed-specific proteins which are close to the predicted molecular weight of the AtS1 and AtS3 gene products. Thus, Western analysis of prebleed and primary antisera from each rabbit indicate that each rabbit produced antibodies against the affinity purified target protein. Furthermore, antisera taken from these immunized rabbits identified a protein in total protein extracts prepared from developing *Arabidopsis* seeds and did not react with total protein extracted from other *Arabidopsis* tissues.

Immunolocalization

Immature seed tissue was prepared and embedded in paraplast in a manner identical to that used for *in situ* localization of Example 3. The paraplast was removed and the tissue rehydrated as described in Example 3. The tissue was treated with 100 mg/mL proteinase K as described above, except the reaction was carried out for 10 minutes at room temperature. The slides were subsequently acetylated as

described above. After two 5 minute rinses in PBS at room temperature, the slides were equilibrated in 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.2% NP-40 for 10 minutes at room temperature. The slides were then incubated in blocking buffer (10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% NP-40, 5% goat serum (Sigma, St. Louis, MO) at room temperature for 1 hour. The primary antiserum was preadsorbed to fixed plant tissue as previously described (Perry et al. (1996) *Plant Cell, 8*; 1977-1989). Hybridization to the primary antiserum was carried out using a 1:100 dilution in blocking buffer at 4°C for at least 12 hours. The unbound antibody was removed through extensive incubations in wash buffer (10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.1% NP-40, 0.1% SDS, 0.25% sodium deoxycholate) over a 12 hour period at room temperature. The detergent was removed by a 20 minute incubation in 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.2% NP-40 at room temperature. The slides were then incubated in blocking solution containing 1:5000 goat anti-rabbit FAB fragment conjugated to alkaline phosphatase for 1 hour at room temperature. They were washed as described for the primary antibody. The hybridization was detected through an alkaline phosphatase reaction.

Light level immunolocalization was used to refine this localization in immature seed tissue. As Figures 13A and 13B indicate, each gene product accumulates in immature embryos. Further, localization corresponds to the cells that express each gene (compare Figures 13A and 13B with Figures 5A-5F and 6A-6F). This data further supports the correct interpretation of the AtS1 and AtS3 structural data and reveals that two novel seed proteins have been identified.

Chromosome Mapping of AtS1

Experiments to determine the map position of both AtS1 and AtS3 were also carried out and were successful in identifying the map position of AtS1. This map position was determined by RFLP analysis of an F2 population segregating from a cross between the WS and HM ecotypes. Inheritance of a *CfoI* polymorphism identified within the AtS1 sequences was correlated with the inheritance of other markers using the Mapmaker computer program (Lander et al. (1987) *Genomics, 1*; 174-181). By this analysis, AtS1 was mapped to the bottom of *Arabidopsis* chromosome 5, approximately 9.2 centimograns above the RFLP marker M558FQ and approximately 2.5

centimorgans below M435F (Kowalski et al. (1994) *Genetics. 138*; 499-510). This is diagramed in Figure 14.

The AtS3 gene has not been chromosome mapped. A polymorphism between the WS and HM ecotypes used in the analysis for the AtS1 gene was not found. A second attempt to map AtS3 gene in the segregating F2 population generated from a cross between Columbia and Landsberg ecotypes was initiated (Lister et al. (1993) *Plant J., 4*; 745-750). This experiment sought to identify a gene specific cleaved amplified polymorphism (CAPS marker; Konieczny et al. (1993) *Plant J., 4*, 403-410) between these two lines and was unsuccessful, even after examining over 80 different restriction enzymes. No attempt to identify another gene specific region was initiated. However, hybridization of an AtS3 gene-specific probe to the ordered bacterial artificial chromosome (BAC) library generated at Texas A&M University (et al. (1995a) *Plant Mol. Biol. Reporter, 13*; 124-128; Choi et al. (1995b) *Weeds World, 2*; 17-20) has identified two BACs (T2N4 and T4F18) which contain the AtS3 gene. This library is being used in an ongoing multinational effort to sequence the *Arabidopsis* genome. One of these BACs, T2N4 has been localized to chromosome 1. Eventually, T2N4 will be mapped and the location of the AtS3 gene determined.

Analysis of the deduced amino acid sequence for AtS1 and AtS3

The largest continuous open reading frame (ORF) for both AtS1 and AtS3 was conceptually translated (Figures 8 and 9, respectively). As indicated earlier, these gene products have not been functionally defined. An *est* representing the AtS1 gene has been identified in an *Arabidopsis* dry seed cDNA library. The GeneBank accession numbers for this *est* clone (cDNA number pap232) are Z20553 and Z29900. Recently, a cDNA with significant similarity to AtS1 was identified in rice. This gene, designated EFA27, was identified as an ABA responsive gene in rice seedlings, and further analysis indicated that it also responds to osmotic stress. It is expressed in developing seeds in a pattern similar to AtS1 expression (Frandsen et al. (1996) *J. Biol. Chem., 271*; 343-348). An alignment of these cDNAs reveals that they are 60.9% identical, and the gene products are 64.4% similar as shown in Figure 15 (Huang et al., 1991). The data in Figure 3-10B also reveals two highly conserved regions that are nearly 100% conserved at the protein level (Frandsen et al., 1996).

This is the only gene identified by AtS1. besides the pap232 clone, in the databases (Altschul et al., 1990). However. database searches with the coding sequence of EFA27 uncover a second *Arabidopsis est* (ATTSO251, GeneBank accession number Z17677). This cDNA is not identified in similar searches using the AtS1 coding sequence. Sequence alignments of ATTS0251 with EFA27 reveal that they are 62.1% identical (Figure 16A). This gene is only 57.5% identical to AtS1 (Figure 16B). These data would argue that EFA27 and AtS1 are related, perhaps members of a small gene family, but they may not be functional homologs of one another.

The AtS3 gene did not match any known sequence. There is no evidence for an AtS3 homolog in the public databases. This gene does not contain a known functional domain as defined by BEAUTY search algorithms (Worley et al. (1995) *Genome Res., 5*; 173-184). However, a Kyte-Doolittle hydrophobicity plot of the putative gene product reveals two very hydrophobic domains, one at the amino terminus and the other at the carboxy terminus (Figure 17A). This may indicate that the AtS3 gene product is embedded in a membrane and may be a receptor or a structural membrane protein.

EXAMPLE 7 HETEROLOGOUS GENE EXPRESSION UNDER CONTROL OF THE AtS1 AND AtS2 PROMOTERS

Construction of transcriptional and translational promoter-GUS fusions

Four expression cassettes based on each gene, AtS1 and AtS3, were constructed. In each expression cassette, the 5'-upstream regulatory region or the 5'-upstream regulatory region along with the 5'-untranslated region were fused to the bacterial *uidA* gene encoding the b-glucuronidase (GUS) enzyme (Jefferson et al., 1987b). These include transcriptional and translational fusions in a pBI101-based binary vector (Figures 18A and 18B). These cassettes utilize the *Agrobacterium* nopaline synthase terminator (NOS terminus) to serve as a transcriptional terminator and polyadenylation signal (Bevan, 1984). The data presented in Examples 2 and 3 indicate that both the AtS1 and AtS3 genes utilize multiple polyadenylation sites and neither contains a consensus polyadenylation signal that might be predicted based on the literature. This is not an unusual situation in the plant kingdom (Li et al. (1995) *Plant*

Mol. Biol., 28; 927-934; Gaubier et al., 1993) and indicates that polyadenylation in plants is not well understood.

b-Glucuronidase (GUS) reporter cassettes used throughout were in pBIN19 (Bevan, 1984; Jefferson (1987a) *Plant Mol. Biol. Reporter, 5*; 387-405). PCR was used to generate each promoter element. To construct the transcriptional fusions, two oligonucleotide primers, 5'-cgcggatcca aagaaagagg cactcgtgag-3' (SEQ ID NO:35) and 5'-gcgcctaggg agtaaagagt ataatg-3' (SEQ ID NO:36) were designed to anneal to the 3' flanking sequence of the AtS3 and AtS1 promoters, respectively, and introduce a *BamHI* restriction site to facilitate cloning. Each primer was then used in conjunction with either the T3 (ATTAACCCTCACTAAAG) SEQ ID NO:35 (AtS3) or T7 primer (AATACGACTCACTATAG) SEQ ID NO:36 (AtS1) in a *Pfu* polymerase reaction to amplify the transcriptional promoter element of each gene with subcloned genomic DNA fragments as template. The reactions contained 2.5 mM each of the 5'- and 3'-primer, 1X *Pfu* polymerase reaction buffer (10mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.75), 2mM $MgSO_4$, 0.1% Triton®X-100, 100 mg/ml BSA), 100 mM each dNTP, and 5 Units *Pfu* polymerase (Stratagene, La Jolla, CA) in a 25 ml reaction. The reactions were subjected to a thermocycle program consisting of a 4.5 minute initial denaturation step followed by 40 cycles of 30 seconds at 95°C, 1 minute at 42°C, and 1 minute at 72°C. This was followed with a 5 minute extension step at 72°C. The reaction products were purified by agarose gel electrophoresis and the QiaquickÔ gel extraction kit (Qiagen, CA).

Transcriptional fusions to the b-glucuronidase reporter gene were constructed using the binary vector pBI01 (Jefferson et al., 1987b). The AtS1 transcriptional fusion (1tsp, Figure 1) was constructed by digesting the AtS1 transcriptional promoter fragment with *SacI* and end-filling with T4 DNA polymerase. The fragment was then digested with *BamHI*. The pBI101 vector was digested with *HindIII*, filled in with Klenow DNA polymerase and digested with *BamHI*. The pBI101 DNA was treated with shrimp alkaline phosphatase according to manufacturer's instructions (Gibco-BRL). The AtS3 transcriptional fusion (3tsp,Figure 1) was constructed by digesting the AtS3 transcriptional promoter with *XbaI* and the pBI101 vector DNA with *XbaI/SmaI*. The pBI101 vector DNA was treated with shrimp alkaline

phosphatase as described above. Both vector and promoter DNA were gel purified as described above, ethanol precipitated and resuspended in 8 ml MQH_2O each. Both the promoter element and vector DNA were combined in a 19 ml reaction containing 1XT4 DNA ligase buffer (50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP, 25 mg/ml BSA) and 10 Units T4 DNA ligase (NEB) and incubated for 12 hours at 15°C. Upon completion, a fraction of this reaction was transformed into the bacterial host, DH10B (Gibco-BRL), by electroporation.

Positive promoter fusions were verified by both restriction and sequence analysis. Mini-prep plasmid DNA was used as templates in cycle sequencing reactions with the SequiTherm cycle sequencing kit (Epicenter Technologies, Madison, WI) or the ABI PRISMÔ dye terminator cycle sequencing kit (Perkin Elmer, Foster City, CA). Sequence analysis was done locally with GCG (Devereux et al., 1984) on a DEC MicroVAXII; database searches were done remotely through NCBI using the BLAST algorithm (Altschul et al., 1990).

To construct the translational fusions, an oligonucleotide primer SEQ ID NO:37 (5'-catgccatgg ctctctctct ttgtctctag actg-3' (AtS1); SEQ ID NO:38 5'-ctagccatgg tacttcagag atttgtgtg-3' (AtS3)) was designed to anneal to the 3'-flanking sequence of each promoter and introduce an *NcoI* restriction site to enable in-frame translational fusions. Each primer was then used in conjunction with either the T3 (AtS3) or T7 primer (AtS1) in a *Pfu* polymerase reaction, as described above, to generate each gene's translational promoter element. The reaction products were gel purified as described above. The translational fusion to the b-glucuronidase reporter gene was achieved by digesting both the vector, NCO-GUS (Maldonado-Mendoza et al. (1996) *Plant Physiol., 110*; 43-49), and insert DNA with *NcoI* and *PstI* (AtSI) or *XbaI* (AtS3). Vector DNA was treated with shrimp alkaline phosphatase as described above. All DNA fragments were gel purified, ligated and transformed into DH10B as described above. Each construct was verified by both restriction and sequence analysis as discussed above. The complete promoter-GUS fusions, including the NOS-terminus, were excised as a *BamHI/EcoRI* fragment (AtS1) and an *XbaI/EcoRI* fragment (AtS3), ligated into the binary vector pBin19 (Bevan, 1984; Jefferson et al., 1987b), and transformed into DH10B as described above.

Transformation of plants with promoter-GUS fusions

The pBin19-based plasmid constructs were used to transform *Arabidopsis thaliana* (cvs. Landsberg erecta or Columbia) and, in some cases, tobacco (*Nicotiana tabacum* cv Xanthi) according to standard procedures (Bechtold et al., 1993; Horsch et al., 1985; Nunberg et al., 1994; Valvekens et al. (1988) *Proc. Natl. Acad. Sci. USA, 85*; 5536-5540). Constructs were transferred into either the LBA4404 or the GV3101 *Agrobacterium tumefaciens* strains. Constructs were then transformed into tobacco leaf discs according to Nunberg et al. (1994) and *Arabidopsis* using either root transformation (Valvekens et al., 1988) or vacuum infiltration (Bechtold et al., 1993). Positive tobacco transformants were selected as described in Nunberg et al. (1994). Positive *Arabidopsis* transformants were selected on media containing 50 mg/mL kanamycin and 600 mg/mL carbenicillin. Regenerated plants were transferred to soil. Transgenic tobacco plants were grown under the optimal conditions described in Nunberg et al. (1994). Plants were self-pollinated, and seeds were regenerated on 400 mg/mL kanamycin (tobacco) or 50 mg/mL kanamycin and 600 mg/mL carbenicillin (*Arabidopsis*). The copy number of each GUS construction integrated into the plant genome was determined by genomic DNA gel blot analysis. GUS activity was analyzed in R2 progeny.

Biochemical and histochemical detection of GUS activity

The standard procedures of Jefferson (1987a) and Jefferson et al. (1987b) as detailed in Bogue et al. (1990) and Nunberg et al. (1994) were followed. Biochemical assays were performed by mixing plant tissue lysates with an equal volume of 2 mM 4-methylumbelliferyl b-D-glucuronide and incubating for 1 hour at 37°C. Fluorometric analyses were done with a minifluorometer (model TKO-100; Hoefer Scientific Instruments, San Francisco, CA) as described previously (Jefferson, 1987a). Protein concentrations were determined by the method of Bradford (1976). Histochemical localizations for GUS activity were determined by incubating whole tissue in 1 mM 5-bromo-4-chloro-3-indolyl glucuronide (X-gluc) as described by Jefferson (1987a) and Jefferson et al. (1987b). The reactions described here were done in the presence of 1 mM potassium ferricyanide and 1 mM potassium ferrocyanide. The X-gluc treatment was carried out for the indicated times at 37°C. Samples were

mounted on microscope slides with 80% glycerol, and visualized by photomicrography using Kodak Ektochrome 160 ASA tungsten film.

The data in Figure 19A demonstrate that the AtS1 promoter (1tsp) is sufficient to confer seed-specific GUS accumulation in transgenic *Arabidopsis*. This activity is quantitively enhanced up to 10-fold when the 5'-UTR is included in the construct (1tlp in Figure 19B). This alteration does not affect the spatial accumulation of GUS activity in the developing embryo (Figures 20A and 20B). In contrast, the data in Figures 19A and 19B reveals that the AtS3 promoter (3tsp) confers little embro-specific GUS accumulation in *Arabidopsis*. The 3tsp expression cassette produces GUS levels slightly above background levels (Figure 19A). In these experiments, background GUS activity is defined as activity measured in non-seed tissue such as leaf. The lower activity of the AtS3 promoter is overcome by the addition of the AtS3 5'-UTR (3tlp, Figure 19). In every case, except 3tsp, the AtS1 and AtS3 expression cassettes confer embryo-specific GUS accumulation in the temporal manner expected (see Figure 1). GUS levels are barely detectable in pre-torpedo stage embryos. GUS activity rapidly rises during the cotyledon stage and remains stable in the dry seed.

The data presented in Figures 19A and 19B demonstrate that elements lying upstream of the AtS1 and AtS3 coding sequence are capable of driving embryo-specific accumulation of GUS activity in transgenic *Arabidopsis*. However, the 3tsp expression cassette does not lead to the accumulation of significant GUS activity whereas 1tsp does (Figures 19B, 20A, 20C and Table 3). Including the promoter's respective 5'-UTR in each expression cassette significantly enhances embryo-specific GUS accumulation (Figures 19, 20B and 20D). The mechanism by which this effect manifests itself may differ between AtS1 and AtS3. It seems clear that the AtS1 5'-UTR has a significant synergistic effect on overall promoter activity.

The native AtS1 promoter (1tsp) is sufficient to confer seed-specific accumulation of GUS activity in both transgenic *Arabidopsis* and transgenic tobacco (Figures 22A through 22D). The 1tsp construct is approximately 55-fold less effective in tobacco when compared to *Arabidopsis*. Addition of the AtS1 5'UTR (1tlp) enhances GUS accumulation up to 23-fold over that of 1tsp (Figure 21B, Table 4). This data indicates that 1tlp is about 28-fold less effective in tobacco.

TABLE 3 COMPARISON OF GUS ACTIVITY LEVELS DRIVEN BY AtS1-AND AtS3-BASED EXPRESSION CASSETTES IN TRANSGENIC ARABIDOPSIS

GUS ACTIVITY[a]		
Construct	**Dry Seed**	**Leaf**
1tsp	1.9±1.0	0.003±0.006
1tlp	22±13	0.019±0.027
3tsp	0.015±0.024	0.003±0.005
3tlp	1.9±1.0	0.014±0.036

[a] Reported as pmoles 4-MU/mg/minute.

TABLE 4 COMPARISON OF GUS ACTIVITY LEVELS DRIVEN BY AtS1-AND AtS3-BASED EXPRESSION CASSETTES IN TRANSGENIC TOBACCO

GUS ACTIVITY[a]		
Construct	**Dry Seed**	**Leaf**
1tsp	0.035±0.026	0.00 ± 0 0
1tlp	0.81±.23	0.015 ±0.007
3tsp	0.002±0.002	0.00 ± 0.0
3tlp	0.32±0.005	0.025 ± 0.012

[a]Reported as pmoles 4-MU/mg/minute.

CLAIMS

1. An isolated nucleic acid comprising a 5' regulatory region from a plant gene which direct seed specific expression, characterized in that the gene is selected from the group consisting in an AtS1 gene or an AtS3 gene.

2. The nucleic acid of claim 1, characterized in that the 5' regulatory region comprises a promoter and a 5' untranslated region.

3. The nucleic acid of one of claims 1 or 2, characterized in that the palnt is *Arabidopsis*.

4. The nucleic acid of claim 3, caracterized in that the AtS1 5' regulatory region is selected from the group consisting of the nucleotide sequence set forth in SEQ ID NO:27, the nucleotide sequence set forth in SEQ ID NO:27 having an insertion, deletion, or substitution of one or more nucleotides, or a contiguous fragment of the nucleotide sequence set forth in SEQ ID NO:27.

5. The nucleic acid of claim 3, characterized in that the AtS3 5' regulatory region is selected from the group consisting of the nucleotide sequence set forth in SEQ ID NO:28, the nucleotide sequence set forth in SEQ ID NO:28 having an insertion, deletion, or substitution of one or more nucleotides, or a contiguous fragment of the nucleotide sequence set forth in SEQ ID NO:28.

6. An isolated nucleic acid comprising a promoter from a plant gene which direct seed specific expression, characterized in that the gene is selected from the group consisting in an AtS1 gene or an AtS3 gene.

7. The nucleic acide of claim 6, charcterized in that the promoter is the untranscribed region consisting of 1.0 to 1.5 kb of 5' upstram sequence of the gene.

8. The nucleic acid of one of claims 6 or 7, characterized in that the plant is *Arabidopsis*.

9. The nucleic acid of claim 8, characterized in that the AtS1 promoter is selected from the group consisting of the nucleotide sequence set forth in SEQ ID NO:23, the nucleotide sequence set forth in SEQ ID NO:23 having an insertion, deletion, or substitution of one or more nucleotides, or a contiguous fragment of the nucleotide sequence set forth in SEQ ID NO:23.

10. The nucleic acid of claim 8, characterized in that the AtS3 promoter is selected from the group consisting of the nucleotide sequence set forth in SEQ ID NO:24, the nucleotide sequence set forth in SEQ ID NO:24 having an insertion, deletion, or substitution of one or more nucleotides, or a contiguous fragment of the nucleotide sequence set forth in SEQ ID NO:24.

11. An isolated nucleic acid comprising a 5' transcribed and untranslated region from a plant gene which directs seed specific expression, characterized in that the gene is selected from the group consisting in an AtS1 gene or an AtS3 gene.

12. The nucleic acid of claim 11, chartacterized in that the plant is *Arabidopsis*.

13. The nucleic acid of claim 12, characterized in that the AtS1 5' transcribed and untranslated region is selected from the group consisting of the nucleotide sequence set forth in SEQ ID NO:25, the nucleotide sequence set forth in SEQ ID NO:25 having an insertion, deletion, or substitution of one or more nucleotides, or a contiguous fragment of the nucleotide sequence set forth in SEQ ID NO:25.

14. The nucleic acid of claim 12, characterized in that the AtS3 5' transcribed and untranslated region is selected from the group consisting of the nucleotide sequence set forth in SEQ ID NO:26, the nucleotide sequence set forth in SEQ ID NO:26 having an insertion, deletion, or substitution of one or more nucleotides, or a contiguous fragment of the nucleotide sequence set forth in SEQ ID NO:26.

15. A plant transformation vector which comprises at least one nucleic acid of any one of Claims 1 to 14.

16. A plant cell comprising at least one nucleic acid of any of Claims 1 to 14.

17. A plant, or progeny of said plant, which has been regenerated from the plant cell of Claim 16.

18. A transgenic plant, or progeny of said plant comprising the nucleic acid of any of Claims 1-14.

19. The plant of one of claims 17 or 18, wherein said plant is a cotton, tobacco, oil seed rape, maize or soybean plant.

20. An expression cassette which comprises at least one 5' regulatory region of any one of claims 1 to 5, operably linked to at least one of a nucleic acid encoding a heterologous gene or a nucleic acid encoding a sequence complementary to a native plant gene.

21. An expression cassette which comprises at least one promoter of any one of claims 6 to 10, operably linked to at least one of a nucleic acid encoding a heterologous gene or a nucleic acid encoding a sequence complementary to a native plant gene.

22. An expression cassette which comprises at least one 5' transcribed and untranslated region of any one of claims 11 to 14, operably linked at its 5' end to a promoter which functions in plants and operably linked at its 3' end to at least one of a nucleic acid encoding a heterologous gene or a nucleic acid encoding a sequence complementary to a native plant gene.

23. The expression cassette of any one of claims 20 to 22, wherein the heterologous gene is at least one of a fatty acid synthesis gene or a lipid metabolism gene.

24. The expression cassette of claim 23 wherein the heterologous gene is selected from the group consisting of an acetyl-coA carboxylase gene, a ketoacyl synthase gene, a malonyl transacylase gene, a lipid desaturase gene, an acyl carrier protein (ACP) gene, a thioesterase gene, an acetyl transacylase gene, or an elongase gene.

25. The expression cassette of claim 23 wherein the lipid desaturase gene is selected from the group consisting of a D6-desaturase gene, a D12-desaturase gene, and a D15-desaturase gene.

26. An expression vector which comprises the expression cassette of any one of claims 20 to 25.

27. A cell comprising the expression cassette of any one of claims 20 to 25.

28. A cell comprising the expression vector of Claim 26.

29. The cell of any one of claims 27 or 28 wherein said cell is a bacterial cell or a plant cell.

30. A transgenic plant comprising the expression cassette of any one of claims 20 to 25.

31. A transgenic plant comprising the expression vector of claim 26.

32. A plant which has been regenerated from the plant cell of any one of claims 27 or 28.

33. The plant of any one of claims 30 to 32, wherein said plant is at least one of a sunflower, soybean, maize, cotton, tobacco, peanut, oil seed rape or *Arabidopisis* plant.

34. Progeny of the plants of any one of claims 30 to 33.

35. Seed from the plant of a,y one of claims 30 to 34.

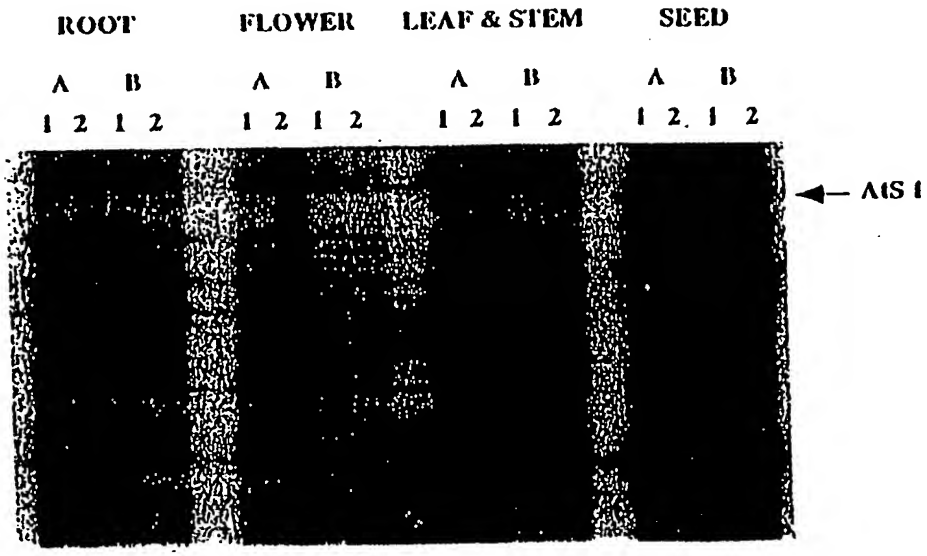


FIGURE 1A

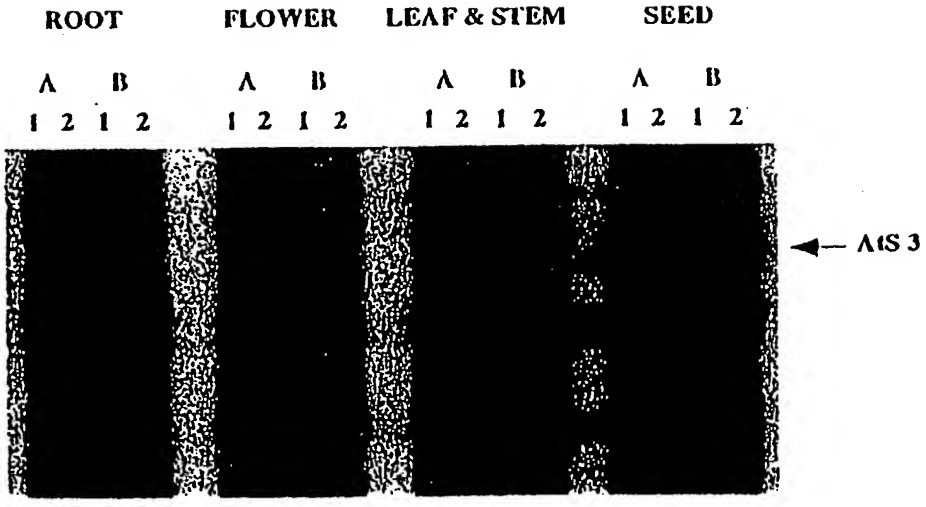


FIGURE 1B

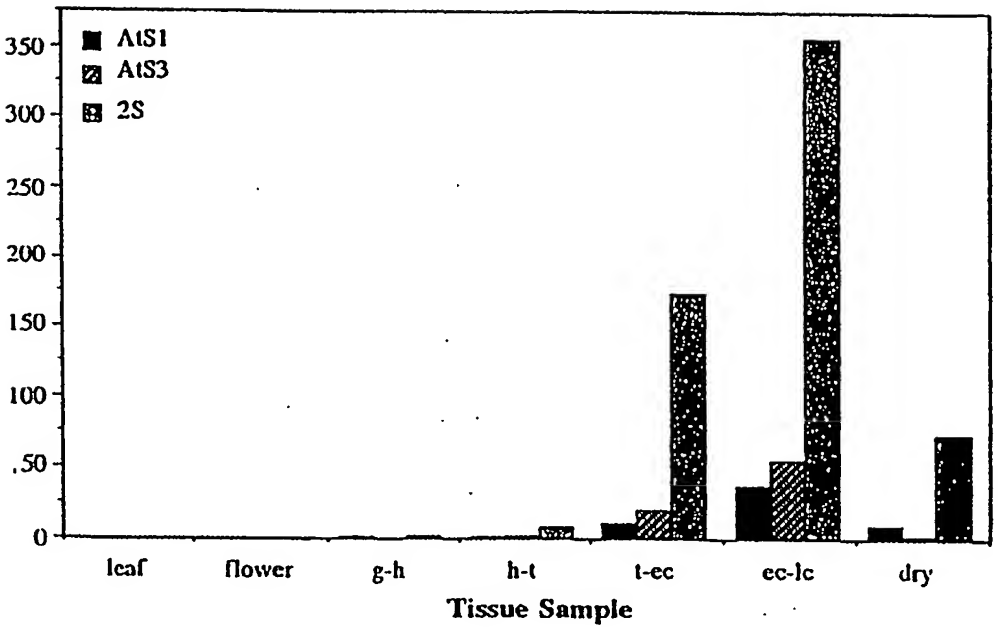

AtS1
AtS3
2S
350
300
250
200
150
100
50
0
leaf
flower
g-h
h-t
t-ec
ec-lc
dry
Tissue Sample

FIGURE 2

AtS1

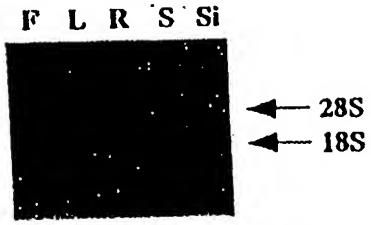


FIGURE 3A

B

AtS3

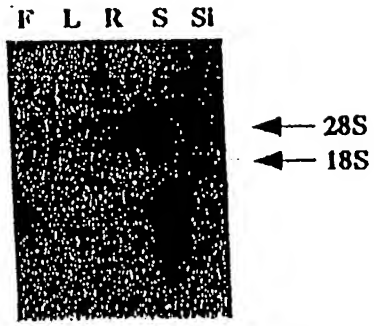


FIGURE 3B

(A) AtS1 cDNA 3'-terminus alignment

```
1-6       ttgcagctct aaagaaaagc ttctgtatgt tttgttgcct tggtctctct   50
1-5       ttgcagctct aaagaaaagc ttctgtatgt tttgttgcct tggtctctct   50
1-4       ttgcagctct aaagaaaagc ttctgtatgt tttgttgccAn---------   39
1-3       ttgcagctct aaagaaaagc ttctgtatgt tttAn----- ----------   33
1-2       ttgcagctct aaagaaaagc ttctgtaAn- ---------- ----------   27
1-1       ttgcagctct aaagaaaaAn ---------- ---------- ----------   18

Genomic   TTGCAGCTCT AAAGAAAAGC TTCTGTATGT TTTGTTGCCT TGGTCTCTCT   50

1-6       ttgtaccaac cccttttctt gttatttcca attttacact gttagttatt  100
1-5       ttgtaccaac cccttttctt gttatttcca attttacact gttagttatt  100
1-4       ---------- ---------- ---------- ---------- ----------   39
1-3       ---------- ---------- ---------- ---------- ----------   33
1-2       ---------- ---------- ---------- ---------- ----------   27
1-1       ---------- ---------- ---------- ---------- ----------   18

Genomic   TTGTACCAAC CCCTTTTTCT GTTATTTCCA ATTTTACACT GTTAGTTATT  100

1-6       attgctaaat ttattactga cttactctaAn    133
1-5       attgctaaat An-------- ---------- ---  110
1-4       ---------- ---------- ---------- ---   39
1-3       ---------- ---------- ---------- ---   33
1-2       ---------- ---------- ---------- ---   27
1-1       ---------- ---------- ---------- ---   18

Genomic   ATTGCTAAAT TTATTACTGA CTTACTCTAA       133
```

FIGURE 4A

(B) AtS3 cDNA 3'terminus alignment

```
3-6     ttacttattc aagtatgtgc gcatgagttc ctgttagcta tgattattaa   50
3-5     ttacttattc aagtatgtgc gcatgagttc ctgttagcta tgaNn-----   43
3-4     ttacttattc aagtatgtgc gcatgagttc ctgttaNn-- ----------   36
3-3     ttacttattc aagtatgtgc gcatgagttc ctgtNn---- ----------   34
3-2     ttacttattc aagtatgtgc gcatgaNn-- ---------- ----------   26
3-1     ttacttattc aagtaNn--- ---------- ---------- ----------   15

Genomic TTACTTNTTC AAGTNTGTGC GCATGAGTTC CTGTTAGCTA TGATTATTAA   50

3-6     atcagttggt accgacaNn 67
3-5     ---------- ------- 43
3-4     ---------- ------- 36
3-3     ---------- ------- 34
3-2     ---------- ------- 26
3-1     ---------- ------- 15

Genomic NTCAGTTGGT ACCGACA  67
```

FIGURE 4B

FIGURE 5B

FIGURE 5D

FIGURE 5F

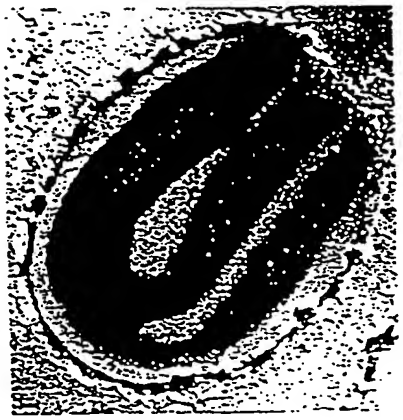


FIGURE 5A

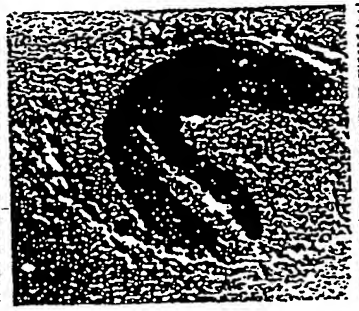


FIGURE 5C

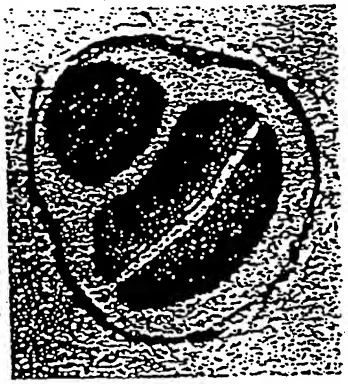


FIGURE 5E

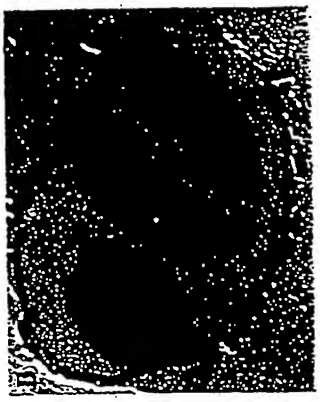


FIGURE 6B

FIGURE 6C

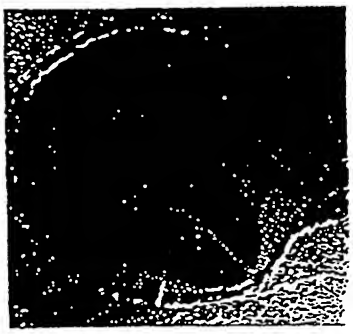


FIGURE 6F

FIGURE 6A

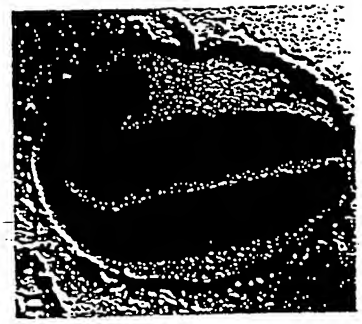


FIGURE 6D

FIGURE 6E

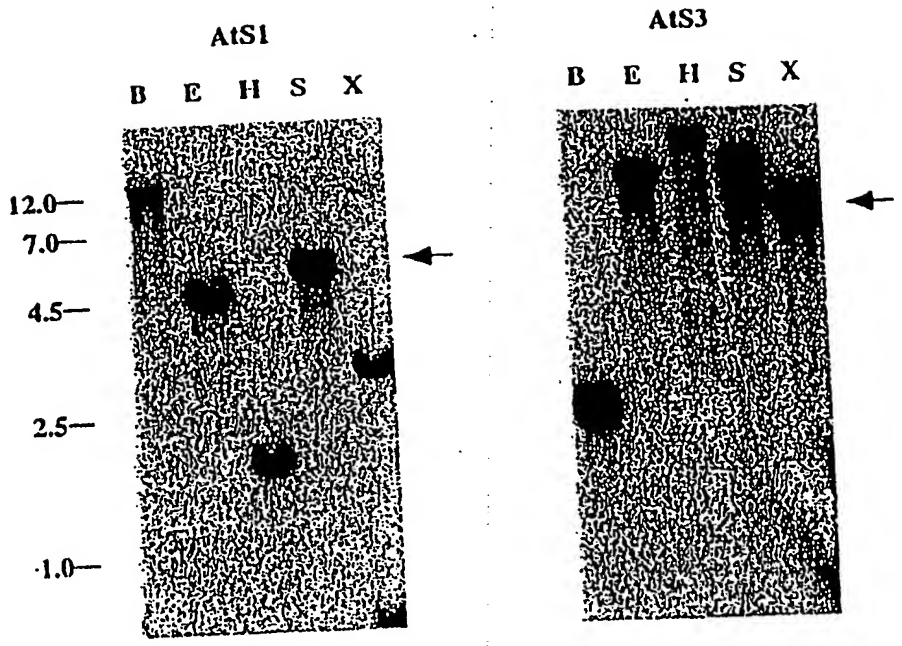


FIGURE 7A

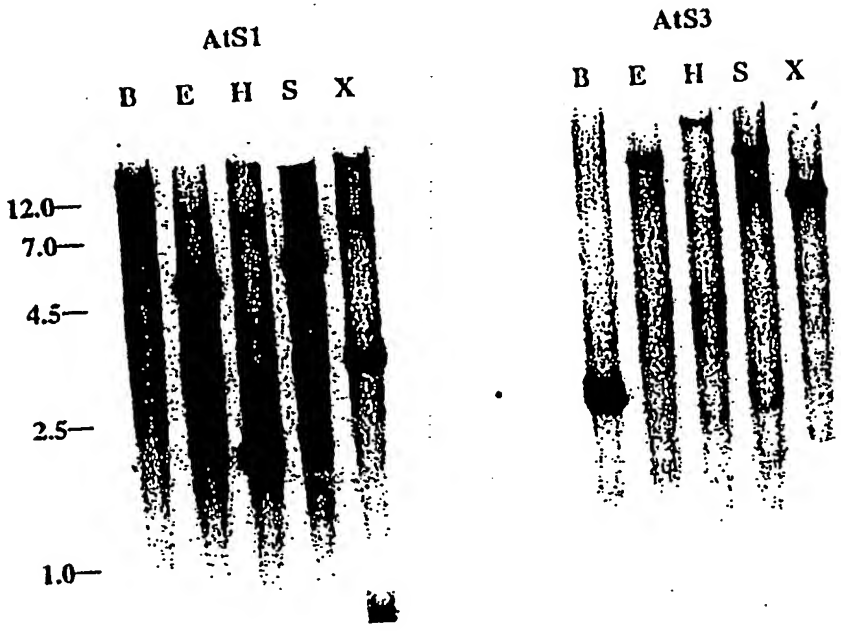


FIGURE 7B

```
cgaattactg aatttagcag acaagaatag aaagagtgat gaaacatgga agaaaacgtg tctctagagt 1050
catgtcaagt gtaagacaga ggaagagaga agagatgtgc gtcaaagaca aggaaagaga gatgtcaatc 1120
gctgctttcg tcggcgcgtg catgtccgcc acgcacatca atcaaatcga tcttattatt attacctcat 1190
tatactcTTT ACTCTAAGAC AAACACATAC ATTTGCACTC AGTCTAGAGA CAAAGAGAGA GAGAGATGGG 1260
                                                              M  G
GTCAAAGACG GAGATGATGG AGAGAGACGC AATGGCTACG GTGGCTCCCT ATGCGCCGGT CACTTACCAT 1330
  S  K  T  E  M  M  E  R  D  A  M  A  T  V  A  P  Y  A  P  V  T  Y  H
CGCCGTGCTC GTGTTGACTT GGATGATAGA CTTCCTAAAC CTTgtaaacc tgtctctcgc tacttgcatt 1400
R  R  A  R  V  D  L  D  D  R  L  P  K  P  Y
ttttatccc taattgattt caatatattg catgccaaaa aacatttgat atatggttga atttaagaaa 1470
ccctttaaa tatatggaat tgccgaccct caaaatttt aaaacatgca tatagaatga tgttcatgat 1540
cttatagaag ctataaattg taaaatgata catatcctgt atatgatggt aattaataat gtattaccca 1610
tgaacgtgca tgaataattc tatacacata ttacacatac gtggaaatga tacagatttt gacttatatg 1680
tgttatgcat agATATGCCA AGAGCATTGC AAGCACCAGA CAGAGAACAC CCGTACGGAA CTCCAGGCCA 1750
                M  P  R  A  L  Q  A  P  D  R  E  H  P  Y  G  T  P  G  H
TAAGAATTAC GGACTTAGTG TTCTTCAACA GCATGTCTCC TTCTTCGATA TCGATGATAA TGGCATCATT 1820
  K  N  Y  G  L  S  V  L  Q  Q  H  V  S  F  F  D  I  D  D  N  G  I  I
TACCCTTGGG AGACCTACTC TGgtatgtct atatagtata tatagatatt tcaacttcaa attttcgtt 1890
Y  P  W  E  T  Y  S  G
agtattatat gtacaaaaag ttgatcccaa ccggtgatta gGACTGCGAA TGCTTGGTTT CAATATCATT 1960
                                  L  R  H  L  G  F  N  I  I
GGGTCGCTTA TAATAGCCGC TGTTATCAAC CTGACCCTTA GCTATGCCAC TCTTCCGgta acacctctcc 2030
G  S  L  I  I  A  A  V  I  N  L  T  L  S  Y  A  T  L  P
tcctctgctg acatatatcg caaaactttg attgatctta ctctagactc ggaaattatc atatccaaat 2100
ccgttgtcca tttgttagt gttctacttg attatatgca gGGGTGGTTA CCTTCACCTT TCTTCCCTAT 2170
                                  G  W  L  P  S  P  F  F  P  I
ATACATACAC AACATACACA AGTCAAAGCA TGGAAGTGAT TCAAAACAT ATGACAATGA AGGAAGgtga 2240
  Y  I  H  N  I  H  K  S  K  H  G  S  D  S  K  T  Y  D  N  E  G  R
gtgaccatat tatcttgaaa aaaacggttg actgatagaa aatatgatga ctgatgcata tggtataact 2310
tccgtatgct tttcagGTTT ATGCCGGTGA ATCTTGAGTT GATATTTAGC AAATATGCGA AAACCTTGCC 2380
            F  M  P  V  N  L  E  L  I  F  S  K  Y  A  K  T  L  P
AGACAAGTTG AGTCTTGGAG AACTATGGGA GATGACAGAA GGAAACCGTG ACGCTTGGGA CATTTTTGGA 2450
  D  K  L  S  L  G  E  L  W  E  M  T  E  G  N  R  D  A  W  D  I  F  G
TGgtacaatc acagcattag ccttcctttt tcttaccctt tcattagttt attgaatgca tgtgttaaac 2520
W
```

FIGURE 8

```
taaagtatta gtcaatgttg ttgtagttat aatgtttgga tctacatgta tgtattagGA TCGCAGGCAA  2590
                                                              I  A  G  K
AATAGAGTGG GGACTGTTGT ACTTGCTAGC AAGGGATGAA GAAGGGTTTT TGTCAAAAGA AGCTATTAGG  2660
  I  E  W  G  L  L  Y  L  L  A  R  D  E  E  G  F  L  S  K  E  A  I  R
CGGTGTTTCG ATGGAAGCTT GTTCGAGTAC TGTGCCAAAA TCTACGCTGG TATCAGTGAA GACAAGACAG  2730
R  C  F  D  G  S  L  F  E  Y  C  A  K  I  Y  A  G  I  S  E  D  K  T  A
CATACTACta aaagtatcct ttatgttaag taattgatcg agccatttta agctaataat cgctcaatgt  2800
  Y  Y
gaagcttgtg cctatacggt aaatgaaggt tcgggtagta gtatggactt ttggtctaag agatctatgt  2870

ttgtttttgt ttttccagtt ctgtatggtt atactataag ttgcagctct aaagaaaagc ttctgtatgt  2940

tttgttgcct tggtctctct ttgtaccaac ccctttttct gttatttcca atttacact gttagttatt  3010
  *  *
attgctaaat ttattactga cttactctat agtagtgtaa cgaatatatg gtcacattaa ctcaaagtta  3080
           *

actccactcc atgaacattg aagcactgag aatccaggac ctatgaatca acgcaatcaa agaaagagaa  3150
agttagtaac acttcatga aggagagtct taaaagaaaa gaagaaaaga ttaaaacacc ttcatgaaag  3220
agagtcttga acttgaatag tatactagtc ctttagagt cttgaagttt gaatagtata ctagttcttt  3290
```

FIGURE 8 (continuation)

```
aaaatgtaag aaggaatagt acaatataga acggtaaaaa aaatggcaaa ccatttactt caataagaaa   1050
ggttagcaac cacactcagc aaatgggaca cataggatcc gacgtggttt atattatagt agtctgatat   1120
tgtagagtca atgggtatat ttgtcttttt caaagactca gtttcactga agagtaggtt acttctttaa   1190
acaagactct gttttgaatg atattgtaaa gttaaggggt acgtttgtct ttttcaggac aaagcgagac   1260
catagatgac gtgtcaactg ctaattttca aaaactcggt ctacaaacca taaggaaact tatttattca   1330
attatttccg tcaaaaaaat ataattttct ttttgcatct caatggattg atcccatgtg ccaagtgttg   1400
gtgttcatga gaaaattagt cgcagctgat gacaacaaac atcaagcatt tataatttat ataacactca   1470
cgAGTGCCTC TTTCTTTATC TACCTCGTCT CCTAATCACA AACACACACA AATCTCTGAA GTAAAATGAC   1540
                                                                        M  T
GTTCCCTTCT CTTTCTGTCT CATTTCTCTT CTTTGCCTTC ATATTCGTTA CGCATGCATT CGACCTCAGC   1610
 F  P  S   L  S  V  S   F  L  F   F  A  F   I  F  V  T   H  A  F   D  L  S
ATCATCCAGg ttcttcttgt tttctacttt ctggctaaca aagtaaccag aaccggtttt ctcacttgta   1680
I  I  Q
tatttgtttt tttgagaaaa tcatgtagAT GCAACAGGGA ACATGTCCGT ACACGGTGGT TGTCATGACA   1750
                              M  Q  Q  G   T  C  P  Y   T  V  V   V  M  T
AGCTGTCTTT CTCCCGAGTC GACAAGAGAT CAGATTAGCA TTGTTTTTGG CGATGCCGAC GGTAACAAGg   1820
S  C  L  S   P  E  S   T  R  D   Q  I  S  I   V  F  G   D  A  D   G  N  K
ttaagtaact agattttttt gtatatagtt ccagttaagt cgacatcttt atttgcttta aagtggttta   1890
gataccttgc atgcatgcat gtgtgctcaa tacaagtaac ttcttagtga tttaaataaa atgttaaata   1960
tatatctttt tgttttagGT GTATGCACCG AAACTAGGGG GTTCGGTAAG AGGACCACGG GGTTTGGGAA   2030
                    V  Y  A  P   K  L  G  G   S  V  R   G  P  G   G  L  G  K
ACTGTTCAAC GAACACATTC CAAGTCAGAG GTCAATGTTT AAATGACCCT ATCTGCTCTC TCTATATCAA   2100
 C  S  T   N  T  F   Q  V  R  G   Q  C  L   N  D  P   I  C  S  L   Y  I  N
CCGGAATGGA CCCGATGGCT GGGTCCCGGA GTCCATTGAG ATCTACTCAG AAGGTTCAAA GTCCGTTAAA   2170
 R  N  G   P  D  G  W   V  P  E   S  I  E   I  Y  S  E   G  S  K   S  V  K
```

FIGURE 9

```
TTCGATTTCA GCAAGAGCGT CCCTCAACTA AACACTTGGT ACGGCCACAA CAACTGCAAC ACCACAGGCA   2240
F D F S   K S V   P Q L   N T W Y   G H N   N C N   T T C R
GACCATCGTC TCCCGATCTG CCTCCACCGC ATTTTCCGCC AGAGTTTCCA CCGGAGACAC CTACCACCCC   2310
  P S S   P D L   P P P H   F P P   E F P   P E T P   T T P.
ACCGCCGCCT CCACCAAGGC CGTCTGCTGC TTCAAGCCTT GGAATGGTG AGAGTGTTTT CCTTGCGTTT   2380
  P P P   P P R P   S A A   S R L   C N G E   S V F   L A F
GCCATTGCGA CTGCGATTGC CGCAATGGTG CGTTGCAGTT ACtagcatgg tacttgaaga gcatgtgtct   2450
A I A T   A I A   A N V   R W S Y
ggattgtatg aggctttttc tttccgtcga atgttttcat ttgcctttcgt ttgcttcag ccttttcccc   2520

gttgtagaaa acataattac ttattcaagt atgtgcgcat gagtccctgt ttagctatga ttattaatca   2590

gttggtaccg acatttagta gttcatttcc aaaagagaat ccatcacttg tgcatagaaa taaagattaa   2660

aaaaatccat cacttccat aaccggtgtt tggacttgca attttttagc gagacagtat gattaatttt   2730
ttttaaagt acatatatgc taatcagtga tccaatttct aacaattgag atgaagattt atccaaaaac   2800
tggtgtatca taccaaatta tcaatagatt atattgagac aaacaaggat ataatttaaa taatttggac   2870
aacaaacttc aacttcaaggc acatttgatg acatttcaag gaaaacataa atggacttaa cttttgattc   2940
gaatcgttat tgaagtgttg tcgaaaactg gaatgcatgg aatttgtcag gtagtagtag gtggagttca   3010
tgggagaagt cgaaacacgt aaacaactct tctctttag acaaatttct tcttttttcg gacatctggt   3080
ttcacgtgtc cttgacctaa aatcgggatt aaatatggct tatattgatg ttacaccgag ccatttttat   3150
ttcctttttac ttaaatcaaa ttgtcttatg atgttaatcc gacaatttct atttattttt actgatttgg   3220
ttttttgagat gttgttcttt taagtcacca taaaattaaa aaaaaaaaaa aaaaagagag agngaaggta   3290
```

FIGURE 9 (continuation)

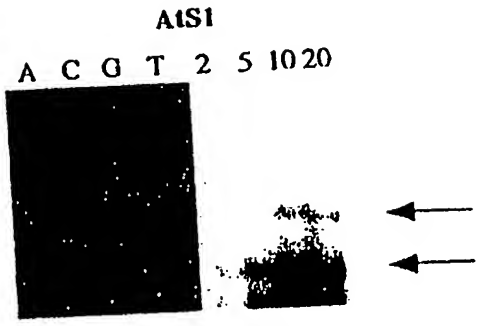


FIGURE 10A

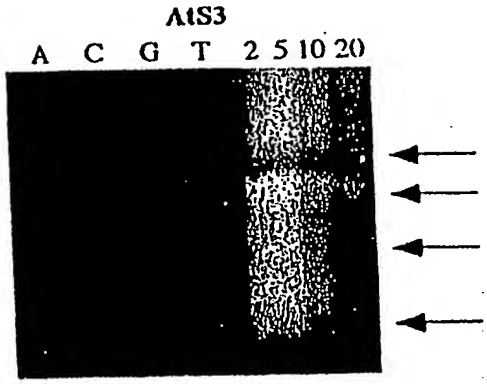


FIGURE 10B

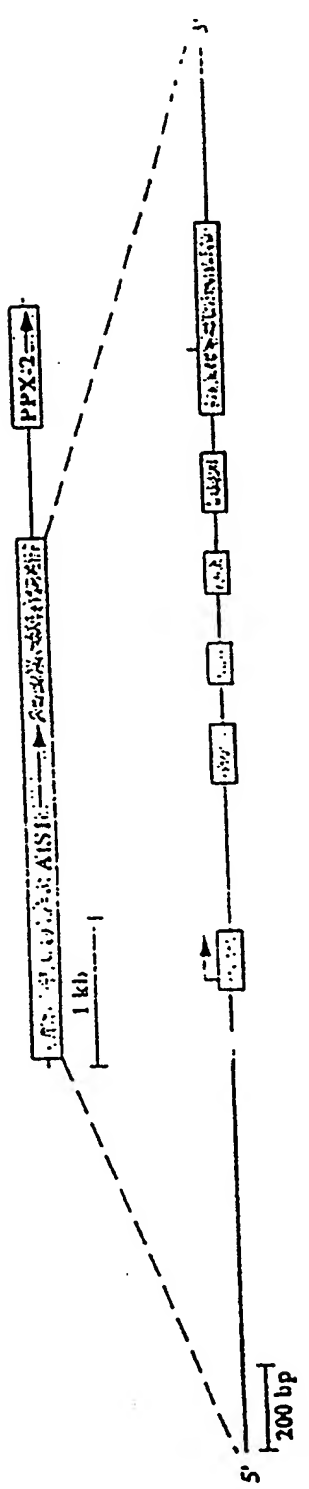
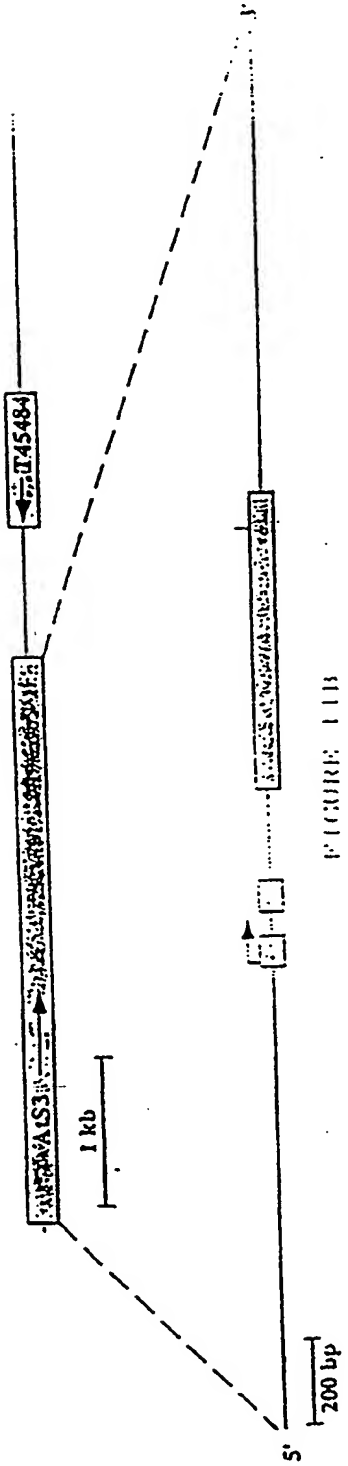


FIGURE 11A



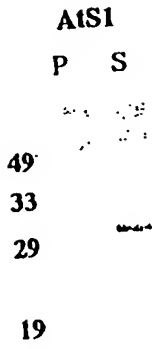


FIGURE 12A

FIGURE 12B

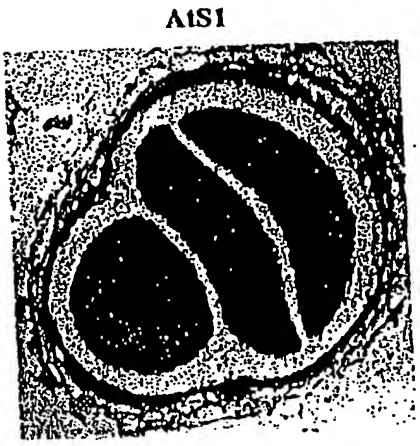


FIGURE 13A

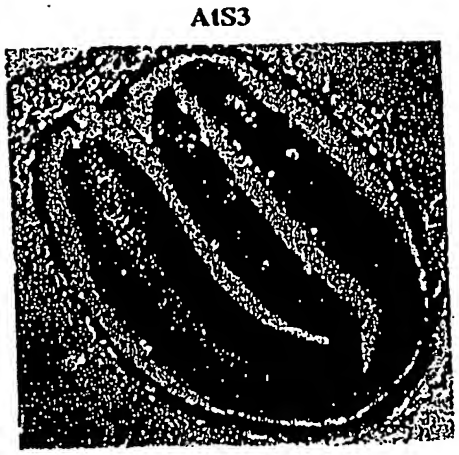


FIGURE 13B

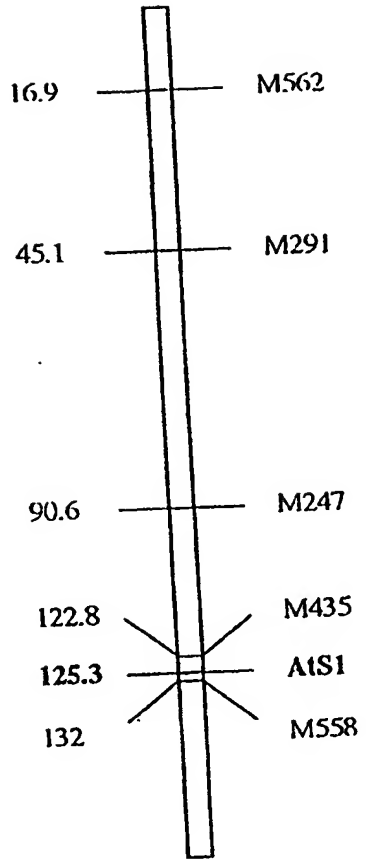


FIGURE 14

60.9% identical
AtS1
EFA27
AtS1
EFA27
AtS1
EFA27
AtS1
EFA27
AtS1
EFA27
AtS1
EFA27
AtS1
EFA27
AtS1
EFA27
ATS1
EFA27

FIGURE 15A

64.4% identity in 222 residue overlap; Score: 765.0; Gap frequency: 0.0%

```
AtS1    23   DAMATVAPYAPVTYIIRRARVDLDDRLPKPYMPRALQARPDREIIPYGTPGIIKNYGLSVLQQII
EFA27   13   DALSSVAAEAPVTRERPVRADLEVQIPKPYLARALVARPDVYIIPEGTEGRDHRQHSVLQQII

AtS1    83   VSFFDIDDNGIIYPWETYSGLRMLGFNIIGSLIIAAVINLTLSYATLPGWLPSPFFPIYI
EFA27   73   VAFFDLDGDGIVYPWETYGGLRELGFNVIVSFFLAIAINVGLSYPTLPSWIPSLLFPIII

AtS1   143   HNIIHKSKIIGSDSKTYDNEGRFMPVNLELIFSKYAKTLPDKLSLGELWEMTEGNRDAWDIF
EFA27  133   KNIIIRAKIIGSDSSTYDNEGRFMPVNFESIFSKNARTAPDKLTFGDIWRMTEGQRVALDLL

AtS1   203   GWIAGKIEWGLLYLLARDESGFLSKEAIRRCFDGSLFEYCAK
EFA27  193   GRIASKGEWILLYVLAKDEEGFLRKEAVRRCFDGSLFESIAQ
```

FIGURE 15B

57.5% identity in 261 nt overlap; init: 177, opt: 258

```
                240        250        260        270        280        290
AtS1   TTCTTCAACAG-CATGTCTCCTTCTTCGATATCGATGATAATGGCATCATTTACCCTTGG
       :::::  : :: X::::: : :: :::::::  :  : ::::  ::: :::: :: : :
ATTS   TTCTTGCAGAGACATGTCGCTTTTTTCGATAGGAACAAAGATGGTATCGTTTATCCCTCG
        180        190        200        210        220        230

                300        310        320        330        340
AtS1   GAGACCTACTCTGGACTGCGAATGCTT-GGTTTCAATATCATTGGGTCGCTTATAATAGC
       :::::  :     ::: :  ::    :: ::: :  ::::   : :   ::    : : ::
ATTS   GAGACATTTCAAGGATTTAGAGCAATTGGGTGTGGATATTTGTTGTCAGC----AGTCGC
        240        250        260        270        280            290

           350        360        370        380        390        400
AtS1   CGCTG---TTATCAACCTGACCCTTAGCTATGCCACTCTTCCGGGGTGGTTACCTTCACC
         :::    : :: ::: :    :: :::       :::: ::::::      :   ::
ATTS   TTCTGTGTTCATAAACATAGGTCTCAGCAGCAAAACTCGTCCGGGTAAAGGATTCTCTAT
            300        310        320        330        340        350

            410        420        430        440        450        460
AtS1   TTTCTTCCCTATATACATACACAACATACACAAGTCAAAGCATGGAAGTGATTCAAAAAC
       :  :: :::::: :  :. : :: :: :::    : :: :: ::::: :::::X
ATTS   CTGGTTTCCTATAGAGGTTAAGAATATTCACCTTGCCAAACACGGAAGCGATTCAGCGT
            360        370        380        390        400        410

            470        480
AtS1   ATATGACAATGAAGGAAGGTT
       :: :::::  :: ::: ::::
ATTS   TTACGACAAAGATGGACGGTT
            420        430
```

FIGURE 16A

```
62.1% identity in 280 nt overlap; init:  223, opt:  362

      270       280       290       300       310       320
EFA27 CCACCGGCAGATGAGTGTGCTGCAGCAGCATGTGGCTTTCTTCGACCTGGATGGCGACGG
      :::   : :::: : : :  :::::   ::::: ::::: :::::   : :    :: X:
ATTS  CCAGAAGAAGAT-AATTTCTTGCAGAGACATGTCGCTTTTTTCGATAGGAACAAAGATGG
            170       180       190       200       210       220

      330       340       350       360       370       380
EFA27 TATCGTTTATCCATGGGAAACTTATGGAGGA-CTACGGGAATTGGGCTTCAACGTGATTG
      :::::::::::: : ::: :: : :  ::::  ::  : :::::::  :  :  :  :::
ATTS  TATCGTTTATCCCTCGGAGACATTTCAAGGATTTAGAGCAATTGGGTGTGGA--TATTTG
            230       240       250       260       270

          390       400       410       420       430       440
EFA27 TT----TCGTTCTTTTTGGCGATAGCCATAAACGTTGGTCTAAGCTACCCAACTCTGCCA
      ::     :  ::  ::  : : :  :::::::  : ::::: :::  :  :::::  ::
ATTS  TTGTCAGCAGTCGCTTCTGTGTT---CATAAACATAGGTCTCAGCAGCAAAACTCGTCCG
      280       290       300           310       320       330

            450       460       470       480       490       500
EFA27 AG--CTGGATACCATCTCTCCTGTTCCCTATACACATAAAAAACATCCACAGGCCTAAGC
       :    :::: :  ::: ::  ::: ::::::  :  : ::  ::  ::  :::   :: :: :
ATTS  GGTAAAGGATTC--TCTATCTGGTTTCCTATAGAGGTTAAGAATATTCACCTTGCCAAAC
        340       350       360       370       380       390

            510       520       530       540
EFA27 ACGGCAGCGATAGCTCGACGTACGACAACGAGGGAAGGTT
      :::: ::::::          :::::::X :: ::: :::
ATTS  ACGGAAGCGATTCAGGCGTTTACGACAAAGATGGACGGTT
          400       410       420       430
```

FIGURE 16B

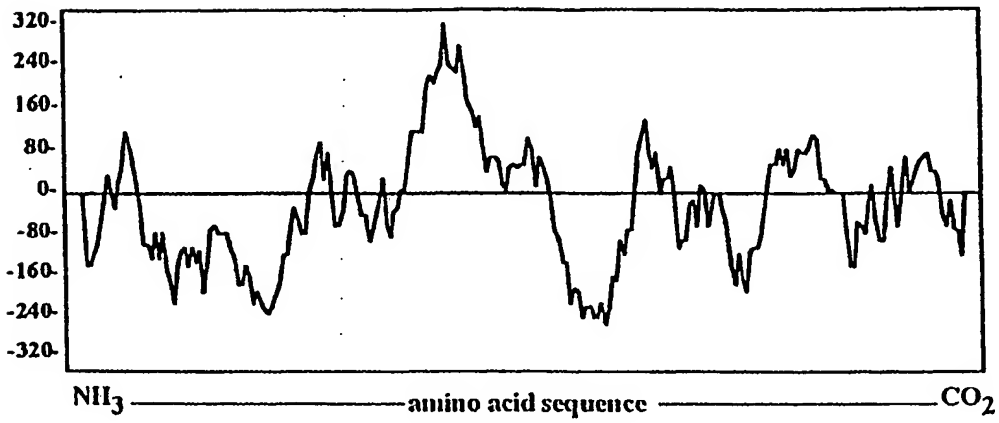


FIGURE 17A

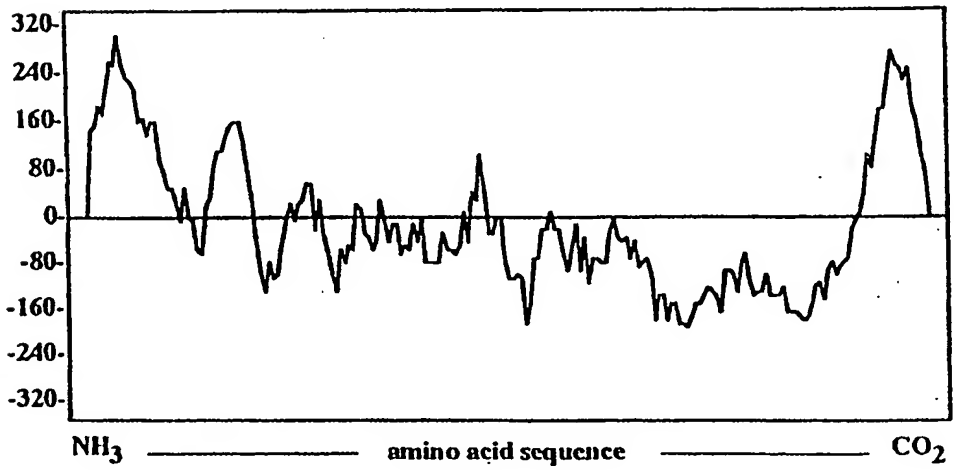


FIGURE 17B

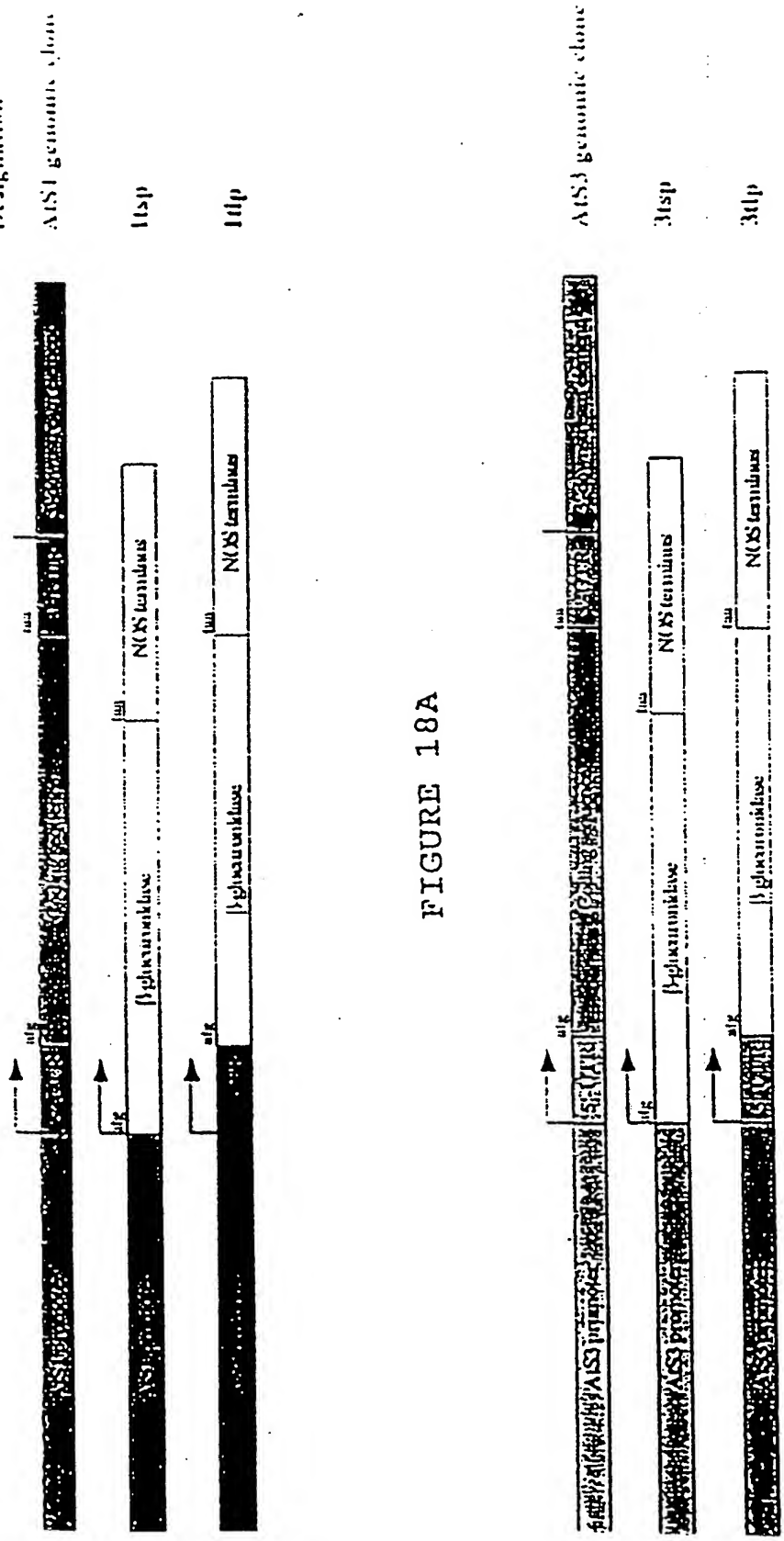


FIGURE 18A

FIGURE 18B

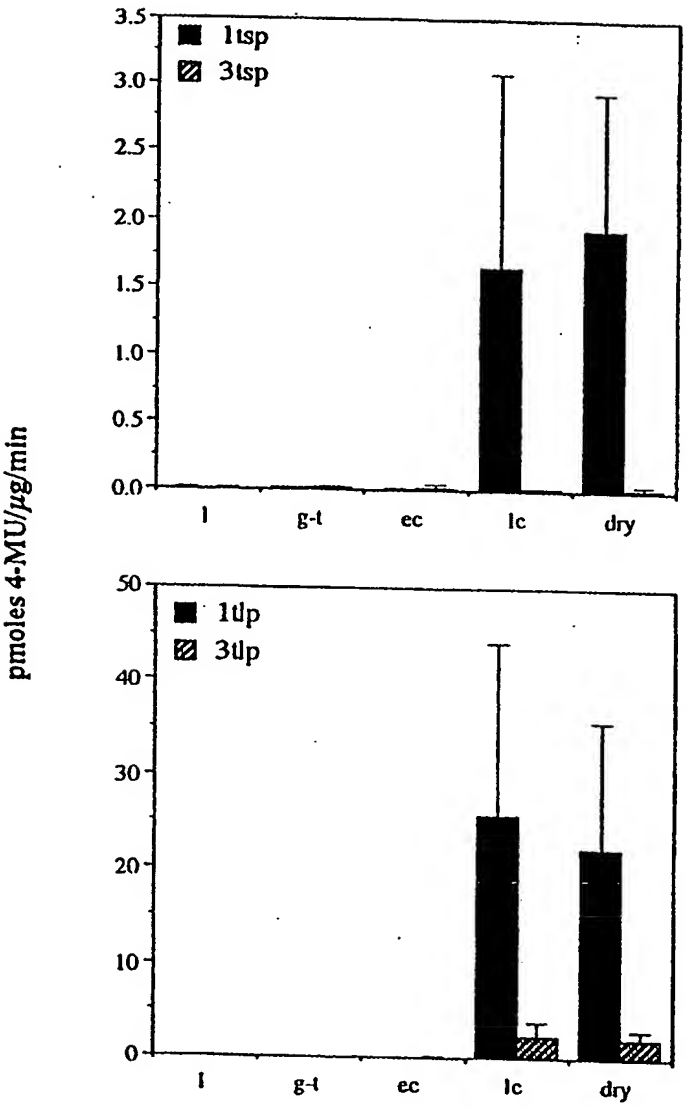


FIGURE 19A

FIGURE 19B

FIGURE 20A

FIGURE 20B

FIGURE 20C

FIGURE 20D

FIGURE 21A

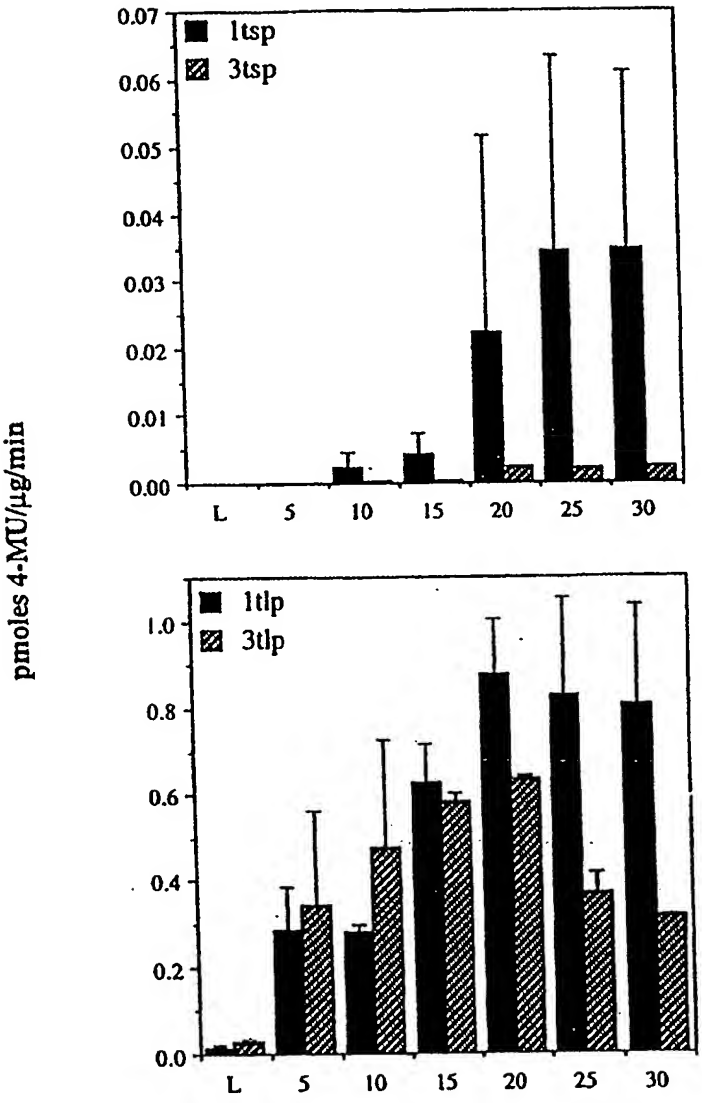


FIGURE 21B

FIGURE 22A

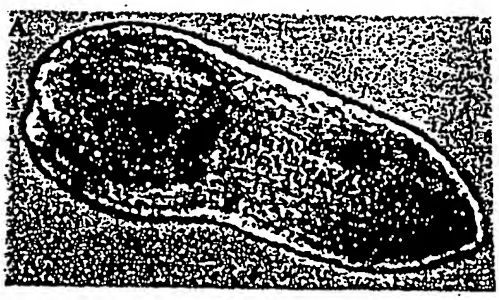


FIGURE 22B

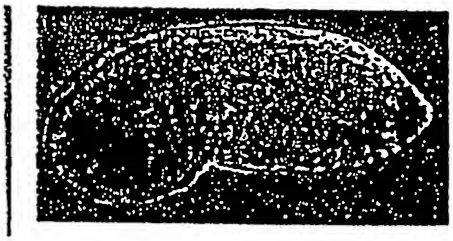


FIGURE 22C

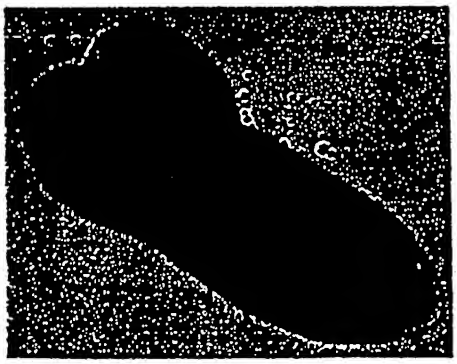


FIGURE 22D

```
                                                aagctCGATCCACCCCATGACTCGAATGATGACTCCAGCCACCA   44

TCAATTCCCTGAAGTGCCACAACACCCTCTACCTCCCAGGTTTTATGACAATCCGACCAACGATTATCCCGCAGATGTCC   124

CACCTCCACCACCGTCTTCTTACCCTTCCAACGATCATCTTCCCCCTCCCACAGGACCATCAGACTCCCCTTACCCGCAT   204

CCTTACAGTCATCAACCATACCACCAAGACCCGCCAAAACACATGCCGCCACCGCAAAACTACTCATCTCATGAGCCTTC   284

TCCAAATTCTCTCCCTAATTTCCAATCTTATCCTAGCTTTAGTGAGAGCAGCCTCCCATCCACTTCTCCCCACTACCCTT   364

CTCACTACCAAAACCCAGAACCTTACTATTCTTCTCCGCACTCTGCACCTGCTCCTTCTTCCACAAGCTTCTGCTCTGCT   444

CCTCCTCCTCCACCTTACTCATCAAACGGGCGTATCAATATTGCTCCCGTGCTAGATCCTGCACCGAGTTCAGCTCAGAA   524

GTACCATTACGATAGCAGCTACCAGCCAGGGCCTGAGAAGGTTGCAGAGGCACTCAAGGCTGCTAGATTCGCTGTGGGAG   604

CTTTGGCTTTTGATGAAGTCTCGACTGCTGTAGAACATCTCAAGAAGTCACTTGAGTTGCTAACAAATCCATCGGCCGGT   684

GCCGGTCACTGAATTTTATATCTAATCTATGACACTTGGGGTTGATGTTAGTGCGTGTGTGTGTTCTCACCACATTTGTG   764

GGTTTGTTTATTAACTTTTCAGGCTCAGACTTCGTTTACAAAGAAAATTTGTGTGAATTATTCTTATTATCATAAAATTT   844

TCCTTGCAACTTCGTGTACATTCATACATACATAGGCAATGGAGTTCCTCTTCAGTCTTCACGTAAAGAGCGAGTGTGGG   924

ACACGCACTCATGTAGCGGGTGGTGTTAGTACTCGAGGTTGGGCCTATATAAAAGCCCATAGAGGCCCGAATTACTGAAT   1004

TTAGCAGACAAGAATAGAAAGAGTGATGAAACATGGAAGAAAACGTGTCTCTAGAGTCATGTCAAGTGTAAGACAGAGGA   1084

AGAGAGAAGAGATGTGCGTCAAAGACAAGGAAAGAGAGATGTCAATCGCTGCTTTCGTCGGCGCGTGCATGTCCGCCACG   1164
                                                +1
CACATCAATCAAATCGATTCTTATTATTATTACCTCATTATACTCTTTACTCCCTAGGCGCGATCCCCGGGTGGTCAGTT   1244

CCTT                                                                               1248
```

FIGURE 23

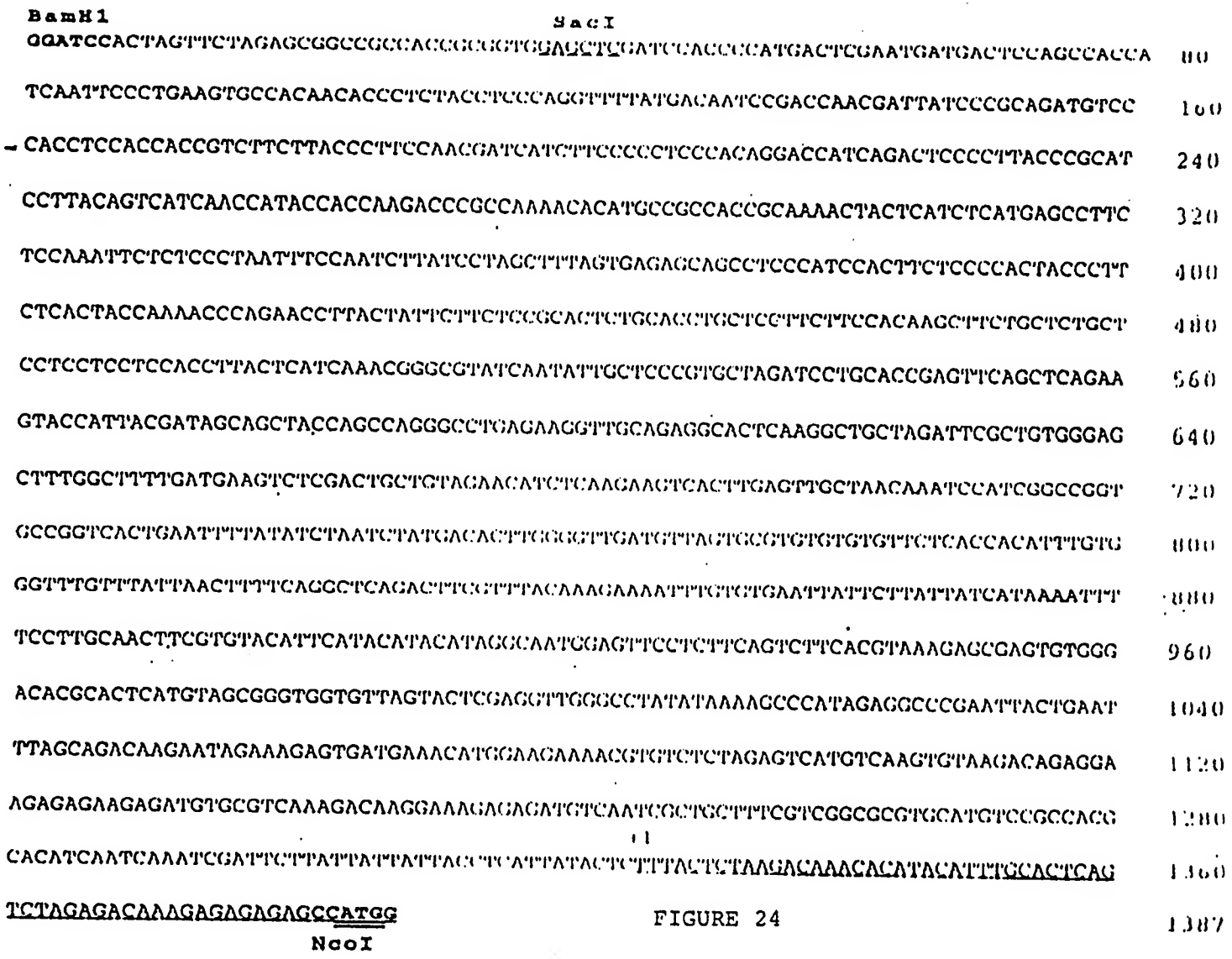

BamH1
SacI
GGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCGATCCACCCCATGACTCGAATGATGACTCCAGCCACCA 80
TCAATTCCCTGAAGTGCCACAACACCCTCTACCTCCCAGGTTTTATGACAATCCGACCAACGATTATCCCGCAGATGTCC 160
CACCTCCACCACCGTCTTCTTACCCTTCCAACGATCATCTTCCCCCTCCCACAGGACCATCAGACTCCCCTTACCCGCAT 240
CCTTACAGTCATCAACCATACCACCAAGACCCGCCAAAACACATGCCGCCACCGCAAAACTACTCATCTCATGAGCCTTC 320
TCCAAATTCTCTCCCTAATTTCCAATCTTATCCTAGCTTTAGTGAGAGCAGCCTCCCATCCACTTCTCCCCACTACCCTT 400
CTCACTACCAAAACCCAGAACCTTACTATTCTTCTCCGCACTCTGCACCTGCTCCTTCTTCCACAAGCTTCTGCTCTGCT 480
CCTCCTCCTCCACCTTACTCATCAAACGGGCGTATCAATATTGCTCCCGTGCTAGATCCTGCACCGAGTTCAGCTCAGAA 560
GTACCATTACGATAGCAGCTACCAGCCAGGGCCTGAGAAGGTTGCAGAGGCACTCAAGGCTGCTAGATTCGCTGTGGGAG 640
CTTTGGCTTTTGATGAAGTCTCGACTGCTGTAGAACATCTCAAGAAGTCACTTGAGTTGCTAACAAATCCATCGGCCGGT 720
GCCGGTCACTGAATTTTTATATCTAATCTATGACACTTCGGGTTGATGTTACTGCCGTGTGTGTTCTCACCACATTTGTG 800
GGTTTGTTTATTAACTTTTCAGGCTCAGACTTCGTTTACAAAGAAAATTTGTGTGAATTATTCTTATTATCATAAAATTT 880
TCCTTGCAACTTCGTGTACATTCATACATACATAGGCAATGGAGTTCCTCTTCAGTCTTCACGTAAAGAGCGAGTGTGGG 960
ACACGCACTCATGTAGCGGGTGGTGTTAGTACTCGAGGTTGGGCCTATATAAAAGCCCATAGAGGCCCGAATTACTGAAT 1040
TTAGCAGACAAGAATAGAAAGAGTGATGAAACATGGAAGAAAACGTGTCTCTAGAGTCATGTCAAGTGTAAGACAGAGGA 1120
AGAGAGAAGAGATGTGCGTCAAAGACAAGGAAAGAGAGATGTCAATCGCTGCTTTCGTCGGCGCGTGCATGTCCGCCACG 1200
CACATCAATCAAATCGATTCTTATTATTATTACCTCCATTATACTCTTTACTCTAAGACAAACACATACATTTGCACTCAG 1360
TCTAGAGACAAAGAGAGAGAGCCATGG 1387
NcoI

FIGURE 24

XbaI

TCTAGATGCATGGGAAGTAATTTTAATTAACCTATGTTTTAAACATTTACATTATTTGGAATTAATATTATATATACACT 80

ATTCGATTTTGTTTTCCTTCAATGTAACATTACTCTGGCAAAAGTATTTATCGTATAATATCTTTTATTATAAATTTTTG 160

ATGTTTTAAAGATTAGTTTATCTCTTTTGACCAAAAAGAAAGGAAAAGGGATTAGATTTATCTCTATGTGAACTTGATTA 240

TACGAGTTCGGATAATCGGATCTCAATGTGATATCCATATTTCTTGCAAGACATATCTCTCGTACACCTTTTATATTTAT 320

ATCCCGCAATCGTGACAACTCTTAATCATTCACTACATAATATTTCCAACAACATTAAAAGATATTTATCTTAATTCTCT 400

TTTCCTTAACACTAACAAAGTAGCATGTCCATATATACTTTCGTTTTTTGAGCATGAGAAAATAGATTTAACTTTATAAG 480

TTATAACCATTGTTTCAAATTAATGCAGATTCGAGTAATAATAATTTGAGATGCAATAATGGTTGTGTCATATCTTGATT 560

GCTAAACTTGATACCGCCATACCGGTAACGTGAAGGGAGAGCTTCCAATTTGTATGCAAGCCTACATCTGACCCAATTGT 640

TGGCCCAATATTAACCAACACCCACACTAAAAAAAAATACTATGGAGGGAGTAATCTACATGCCTACATTCCAAAGCAGGC 720

AATATCGTTTTTTCATGTCTGAAAACGCAATTTTTTTTTCTAATTGTTAAGTTGGTTCAAAAGAAATGAACATGGGTAAT 800

AATAAAAATGATGTATTTGTTTGCAAACAGCAGTTCTCACTTGTCTCTCTCTATATGATGAAAGACAATGTTGTAATCTT 880

FIGURE 25

TATAGGTTTCAATATAGCGGGTATACTTGGTGACATAAAGCGTTATGAAATTTTAAGCAGTAAATAGGAAATGATAAATG 960

ATTATTAAATTCGTTATTAAAAATGTAAGAAGGAATAGTACAATATAGAACGGTAAAAAAAAATGGCAAACCATTTACTTC 1040

AATAAGAAAGGTTAGCAACCACACTCAGCAAATGGGACACATAGGATCCGACGTGGTTTATATTATAGTAGTCTGATATT 1120

GTAGAGTCAATGGGTATATTTGTCTTTTTCAAAGACTCAGGTTCCATTGAAGCGTTACGTTCTTTAAACAAGACTCTG 1200

TTTTTGAATGATATTGTAAGTTAAGGGGTACGTTTGTCTTTTTTCAGGACAAAGCGAGACCATAGATGACGTGTCAACTGC 1280

TAATTTTCAAAAACTCGGTCTACAAACCATAACCAAACTTATTTATTTCAATTATTTTCCGTCAAAAAAATATAATTTTCTT 1360

TTTGCATCTCAATGGATTGATTCCATGTGCCAAGTGTTGGTGTTCATGAGAAATTTAGTCGCAGCTGATGACAACAAACA 1440

+1

TCAAGCATTTATAATTTATATAACACTCACGAGTGCCTCTTTCTTTTGGATCCGCGGGGTGGTCAGTTCCTT 1510

gagtgctcacggagaaagaaacctaggcgc5'

FIGURE 25 (continuation)

XbaI
TCTAGATGCATGGGAAGTAATTTTAATTAACCTATGTTTTAAACATTTACATTATTTGGAATTAATATTATATATACACT 80

ATTCGATTTTGTTTTCCTTCAATGTAACATTACTCTGGCAAAAGTATTTATCGTATAATATCTTTTATTATAAATTTTTG 160

ATGTTTTAAAGATTAGTTTATCTCTTTTGACCAAAAAGAAAGGAAAAGGGATTAGATTTATCTCTATGTGAACTTGATTA 240

TACGAGTTCGGATAATCGGATCTCAATGTGATATCCATATTTCTTGCAAGACATATCTCTCGTACACCTTTTATATTTAT 320

ATCCCGCAATCGTGACAACTCTTAATCATTCACTACATAATATTTCCAACAACATTAAAAGATATTTATCTTAATTCTCT 400

TTTCCTTAACACTAACAAAGTAGCATGTCCATATATACTTTCGTTTTTTGAGCATGAGAAAATAGATTTAACTTTATAAG 480

TTATAACCATTGTTTCAAATTAATGCAGATTCGAGTAATAATAATTTGAGATGCAATAATGGTTGTGTCATATCTTGATT 560

GCTAAACTTGATACCGCCATACCGGTAACGTGAAGGGAGAGCTTCCAATTTGTATGCAAGCCTACATCTGACCCAATTGT 640

TGGCCCAATATTAACCAACACCCACACTAAAAAAAAATACTATGGAGGGAGTAATCTACATGCCTACATTCCAAAGCAGGC 720

AATATCGTTTTTTCATGTCTGAAAACGCAATTTTTTTTTCTAATTGTTAAGTTGGTTCAAAAGAAATGAACATGGGTAAT 800

AATAAAAATGATGTATTTGTTTGCAAACAGCAGTTCTCACTTGTCTCTCTCTATATGATGAAAGACAATGTTGTAATCTT 880

FIGURE 26

```
TATAGGTTTCAATATAGCGGGTATACTTGGTGACATAAAGCGTTATGAAATTTTAAGCAGTAAATAGGAAATGATAAATG    960

ATTATTAAATTCGTTATTAAAAATGTAAGAAGGAATAGTACAATATAGAACGGTAAAAAAAATGGCAAACCATTTACTTC   1040

AATAAGAAAGGTTAGCAACCACACTCAGCAAATGGGACACATAGGATCCGACGTGGTTTATATTATAGTAGTCTGATATT   1120

GTAGAGTCAATGGGTATATTTGTCTTTTTCAAAGACTCAGTTCCATTGAAGCGTAGGTTACTTCTTTAAACAAGACTCTG   1200

TTTTGAATGATATTGTAAAGTTAAGGGGTACGTTTGTCTTTTTCAGGACAAAGCGAGACCATAGATGACGTGTCAACTGC   1280

TAATTTTCAAAAACTCGGTCTACAAACCATAACCAAACTTATTTATTCAATTATTTCCGTCAAAAAAATATAATTTTCTT   1360

TTTGCATCTCAATGGATTGATTCCATGTGCCAAGTGTTGGTGTTCATGAGAAAATTAGTCGCAGCTGATGACAACAAACA   1440

                                    +1
TCAAGCATTTATAATTTATATAACACTCACGAGTGCCTCTTTCTTTATCTACCTCGTCTCCTAATCACAAACACACACAA   1520

ATCTCTGAAGTACCATGG                                                                  1537
      NcoI
```

FIGURE 26 (continuation)

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: RHONE POULENC AGRO.

(ii) TITLE OF INVENTION: NOVEL SEED SPECIFIC PROMOTERS BASED ON PLANT GENES

(iii) NUMBER OF SEQUENCES: 42

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: 10/20 rue Pierre Baizet
(C) CITY: Lyon
(E) COUNTRY: France
(F) ZIP: 69370

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTGCAGCTCT AAAGAAAA 18

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTGCAGCTCT AAAGAAAAGC TTCTGTA 27

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTGCAGCTCT AAAGAAAAGC TTCTGTATGT TTT 33

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTGCAGCTCT AAAGAAAAGC TTCTGTATGT TTTGTTGCC 39

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 110 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTGCAGCTCT AAAGAAAAGC TTCTGTATGT TTTGTTGCCT TGGTCTCTCT TTGTACCAAC 60

CCCTTTTTCT GTTATTTCCA ATTTTACACT GTTAGTTATT ATTGCTAAAT 110

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 129 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TTGCAGCTCT AAAGAAAAGC TTCTGTATGT TTTGTTGCCT TGGTCTCTCT TTGTACCAAC 60

CCCTTTTTCT GTTATTTCCA ATTTTACACT GTTAGTTATT ATTGCTAAAT TTATTACTGA 120

CTTACTCTA 129

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTACTTATTC AAGTA 15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTACTTATTC AAGTATGTGC GCATGA 26

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTACTTATTC AAGTATGTGC GCATGAGTTC CTGT 34

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTACTTATTC AAGTATGTGC GCATGAGTTC CTGTTA 36

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTACTTATTC AAGTATGTGC GCATGAGTTC CTGTTAGCTA TGA 43

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTACTTATTC AAGTATGTGC GCATGAGTTC CTGTTAGCTA TGATTATTAA ATCAGTTGGT 60

ACCGACA 67

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2310 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGAATTACTG AATTTAGCAG ACAAGAATAG AAAGAGTGAT GAAACATGGA AGAAAACGTG 60

TCTCTAGAGT CATGTCAAGT GTAAGACAGA GGAAGAGAGA AGAGATGTGC GTCAAAGACA 120

AGGAAAGAGA GATGTCAATC GCTGCTTTCG TCGGCGCGTG CATGTCCGCC ACGCACATCA 180

ATCAAATCGA TCTTATTATT ATTACCTCAT TATACTCTTT ACTCTAAGAC AAACACATAC 240

ATTTGCACTC AGTCTAGAGA CAAAGAGAGA GAGAGATGGG GTCAAAGACG GAGATGATGG 300

AGAGAGACGC AATGGCTACG GTGGCTCCCT ATGCGCCGGT CACTTACCAT CGCCGTGCTC 360

GTGTTGACTT GGATGATAGA CTTCCTAAAC CTTGTAAACC TGTCTCTCGC TACTTGCATT 420

TTTTTATCCC TAATTGATTT CAATATATTG CATGCCAAAA AACATTTGAT ATATGGTTGA 480

ATTTAAGAAA CCCTTTTAAA TATATGGAAT TGCCGACCCT CAAAATTTTT AAAACATGCA 540

TATAGAATGA TGTTCATGAT CTTATAGAAG CTATAAATTG TAAAATGATA CATATCCTGT 600

ATATGATGGT AATTAATAAT GTATTACCCA TGAACGTGCA TGAATAATTC TATACACACA 660

TTACACATAC GTGGAAATGA TACAGATTTT GACTTATATG TGTTATGCAT AGATATGCCA 720

AGAGCATTGC AAGCACCAGA CAGAGAACAC CCGTACGGAA CTCCAGGCCA TAAGAATTAC 780

GGACTTAGTG TTCTTCAACA GCATGTCTCC TTCTTCGATA TCGATGATAA TGGCATCATT 840

TACCCTTGGG AGACCTACTC TGGTATGTCT ATATAGTATA TATAGATATT TCAACTTCAA 900

ATTTTTCGTT AGTATTATAT GTACAAAAAG TTGATCCCAA CCGGTGATTA GGACTGCGAA 960

TGCTTGGTTT CAATATCATT GGGTCGCTTA TAATAGCCGC TGTTATCAAC CTGACCCTTA 1020

GCTATGCCAC TCTTCCGGTA ACACCTCTCC TCCTCTGCTG ACATATATCG CAAAACTTTG 1080

ATTGATTCTA CTCTAGACTC GGAAATTATC ATATCCAAAT CCGTTGTCCA TTTTGTTAGT 1140

GTTCTACTTG ATTATATGCA GGGGTGGTTA CCTTCACCTT TCTTCCCTAT ATACATACAC 1200

AACATACACA AGTCAAAGCA TGGAAGTGAT TCAAAAACAT ATGACAATGA AGGAAGGTGA 1260

GTGACCATAT TATCTTGAAA AAAACGGTTG ACTGATAGAA AATATGATGA CTGATGCATA 1320

TGGTATAACT TCCGTATGCT TTTCAGGTTT ATGCCGGTGA ATCTTGAGTT GATATTTAGC 1380

AAATATGCGA AAACCTTGCC AGACAAGTTG AGTCTTGGAG AACTATGGGA GATGACAGAA 1440

GGAAACCGTG ACGCTTGGGA CATTTTTGGA TGGTACAATC ACAGCATTAG CCTTCCTTTT 1500

TCTTACCCTT TCATTAGTTT ATTGAATGCA TGTGTTAAAC TAAAGTATTA GTCAATGTTG 1560

TTGTAGTTAT AATGTTTGGA TCTACATGTA TGTATTAGGA TCGCAGGCAA AATAGAGTGG 1620

GGACTGTTGT ACTTGCTAGC AAGGGATGAA GAAGGGTTTT TGTCAAAAGA AGCTATTAGG 1680

CGGTGTTTCG ATGGAAGCTT GTTCGAGTAC TGTGCCAAAA TCTACGCTGG TATCAGTGAA 1740

GACAAGACAG CATACTACTA AAAGTATCCT TTATGTTAAG TAATTGATCG AGCCATTTTA 1800

AGCTAATAAT CGCTCAATGT GAAGCTTGTG CCTATACGGT AAATGAAGGT TCGGGTAGTA 1860

GTATGGACTT TTGGTCTAAG AGATCTATGT TTGTTTTTGT TTTTCCAGTT CTGTATGGTT 1920

ATACTATAAG TTGCAGCTCT AAAGAAAAGC TTCTGTATGT TTTGTTGCCT TGGTCTCTCT 1980

TTGTACCAAC CCCTTTTTCT GTTATTTCCA ATTTTACACT GTTAGTTATT ATTGCTAAAT 2040

TTATTACTGA CTTACTCTAT AGTAGTGTAA CGAATATATG GTCACATTAA CTCAAAGTTA 2100

ACTCCACTCC ATGAACATTG AAGCACTGAG AATCCAGGAC CTATGAATCA ACGCAATCAA 2160

AGAAAGAGAA AGTTAGTAAC ACCTTCATGA AGGAGAGTCT TAAAAGAAAA GAAGAAAAGA 2220

TTAAAACACC TTCATGAAAG AGAGTCTTGA ACTTGAATAG TATACTAGTC CTTTTAGAGT 2280

CTTGAAGTTT GAATAGTATA CTAGTTCTTT 2310

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2310 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AAAATGTAAG AAGGAATAGT ACAATATAGA ACGGTAAAAA AAATGGCAAA CCATTTACTT 60

CAATAAGAAA GGTTAGCAAC CACACTCAGC AAATGGGACA CATAGGATCC GACGTGGTTT 120

ATATTATAGT AGTCTGATAT TGTAGAGTCA ATGGGTATAT TTGTCTTTTT CAAAGACTCA 180

GTTCCATTGA AGCGTAGGTT ACTTCTTTAA ACAAGACTCT GTTTTGAATG ATATTGTAAA 240

GTTAAGGGGT ACGTTTGTCT TTTTCAGGAC AAAGCGAGAC CATAGATGAC GTGTCAACTG 300

CTAATTTTCA AAAACTCGGT CTACAAACCA TAAGGAAACT TATTTATTCA ATTATTTCCG 360

TCAAAAAAAT ATAATTTTCT TTTTGCATCT CAATGGATTG ATTCCATGTG CCAAGTGTTG 420

GTGTTCATGA GAAAATTAGT CGCAGCTGAT GACAACAAAC ATCAAGCATT TATAATTTAT 480

ATAACACTCA CGAGTGCCTC TTTCTTTATC TACCTCGTCT CCTAATCACA AACACACACA 540

AATCTCTGAA GTAAAATGAC GTTCCCTTCT CTTTCTGTCT CATTTCTCTT CTTTGCCTTC 600

ATATTCGTTA CGCATGCATT CGACCTCAGC ATCATCCAGG TTCTTCTTGT TTTCTACTTT 660

CTGGCTAACA AAGTAACCAG AACCGGTTTT CTCACTTGTA TATTTGTTTT TTTGAGAAAA 720

TCATGTAGAT GCAACAGGGA ACATGTCCGT ACACGGTGGT TGTCATGACA AGCTGTCTTT 780

CTCCGGAGTC GACAAGAGAT CAGATCAGCA TTGTTTTTGG CGATGCCGAC GGTAACAAGG 840

TTAAGTAACT AGATTTTTTT GTATATAGTT CCAGTTAAGT CGACATCTTT ATTTGCTTTA 900

AAGTGGTTTA GATACCTTGC ATGCATGCAT GTGTGCTCAA TACAAGTAAC TTCTTAGTGA 960

TTTAAATAAA ATGTTAAATA TATATCTTTT TGTTTTAGGT GTATGCACCG AAACTAGGGG 1020

GTTCGGTAAG AGGACCAGGG GGTTTGGGAA AGTGTTCAAC GAACACATTC CAAGTCAGAG 1080

GTCAATGTTT AAATGACCCT ATCTGCTCTC TCTATATCAA CCGGAATGGA CCCGATGGCT 1140

GGGTCCCGGA GTCCATTGAG ATCTACTCAG AAGGTTCAAA GTCCGTTAAA TTCGATTTCA 1200

GCAAGAGCGT CCCTCAACTA AACACTTGGT ACGGCCACAA CAACTGCAAC ACCACAGGCA 1260

GACCATCGTC TCCCGATCTG CCTCCACCGC ATTTTCCGCC AGAGTTTCCA CCGGAGACAC 1320

CTACCACCCC ACCGCCGCCT CCACCAAGGC CGTCTGCTGC TTCAAGGCTT GGAAATGGTG 1380

AGAGTGTTTT CCTTGCGTTT GCCATTGCGA CTGCGATTGC CGCAATGGTG CGTTGGAGTT 1440

ACTAGCATGG TACTTGAAGA GCATGTTGTT GGGTTGTATG AGGCTTTTTC TTTCCGTCGA 1500

ATGTTTTTAT TTGCTTTCGT TTTGCTTCAG CCTTTTCCTT GTTGTAGAAA ACATAATTAC 1560

TTATTCAAGT ATGTGCGCAT GAGTTCCTGT TTAGCTATGA TTATTAATCA GTTGGTACCG 1620

ACATTTAGTA GTTCATTTTC AAAAGAGAAT CCATCACTTG TGCATAGAAA TAAAGATTAA 1680

AAAAATCCAT CACTTTCCAT AACCGGTGTT TGGACTTGCA ATTTTTTAGC GAGACAGTAT 1740

GATAATTTTT TTTTTAAAGT ACATATATGC TAATCAGTGA TCCAATTTTT AACAATTGAG 1800

ATGAAGATTT ATCCAAAAAC TGGTGTATCA TACCAAATTA TCAATAGATT ATATTGAGAC 1860

AAACAAGGAT ATAATTTAAA TAATTTGGAC AACAAACCTC AACTCAAGGC ACATTTGATG 1920

ACATTTCAAG GAAAACATAA ATGGACCTAA CTTTTGATTC GAATTGTTAT TGAAGTGTTG 1980

TCGAAAACTG GAATGCATGG AATTTGTCAG GTAGTAGTAG GTGGAGTTCA TGGGAGAAGT 2040

CGAAACACGT AAACAACTCT TCTCTTTTAG ACAAATTTCT TCTTTTTTCG GACATCTGGT 2100

TTCACGTGTC CTTGACCTAA AATCGGGATT AAATATGGCT TATATTGATG TTACACCGAG 2160

CCATTTTCAT TTTCTTTTAC TTAAATCAAA TTGTCTATTG ATGTTAATCC GACAATTTTT 2220

ATTTTATTTT ACTGATTTTG TTTTTGAGAT GTTGTTCTTT TAAGTCACCA TAAAATTAAA 2280

AAAAAAAAAA AAAAAGAGAG AGAGAAGGTA 2310

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 735 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATGGGGTCAA AGACGGAGAT GATGGAGAGA GACGCAATGG CTACGGTGGC TCCCTATGCG 60

CCGGTCACTT ACCATCGCCG TGCTCGTGTT GACTTGGATG ATAGACTTCC TAAACCTTAT 120

ATGCCAAGAG CATTGCAAGC ACCAGACAGA GAACACCCGT ACGGAACTCC AGGCCATAAG 180

AATTACGGAC TTAGTGTTCT TCAACAGCAT GTCTCCTTCT TCGATATCGA TGATAATGGC 240

ATCATTTACC CTTGGGAGAC CTACTCTGGA CTGCGAATGC TTGGTTTCAA TATCATTGGG 300

TCGCTTATAA TAGCCGCTGT TATCAACCTG ACCCTTAGCT ATGCCACTCT TCCGGGGTGG 360

TTACCTTCAC CTTTCTTCCC TATATACATA CACAACATAC ACAAGTCAAA GCATGGAAGT 420

GATTCAAAAA CATATGACAA TGAAGGAAGG TTTATGCCGG TGAATCTTGA GTTGATATTT 480

AGCAAATATG CGAAAACCTT GCCAGACAAG TTGAGTCTTG GAGAACTATG GGAGATGACA 540

GAAGGAAACC GTGACGCTTG GGACATTTTT GGATGGATCG CAGGCAAAAT AGAGTGGGGA 600

CTGTTGTACT TGCTAGCAAG GGATGAAGAA GGGTTTTTGT CAAAAGAAGC TATTAGGCGG 660

TGTTTCGATG GAAGCTTGTT CGAGTACTGT GCCAAAATCT ACGCTGGTAT CAGTGAAGAC 720

AAGACAGCAT ACTAC 735

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 732 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATGGCGGAGG AGGCGGCTAG CAAGGCAGCG CCGACCGATG CGCTGTCGTC CGTGGCGGCG 60

GAGGCGCCGG TGACGAGAGA ACGGCCGGTC CGAGCGGACT TGGAAGTGCA GATTCCGAAG 120

CCCTATTTGG CCCGAGCTCT GGTTGCTCCG GACGTGTACC ATCCTGAAGG AACCGAGGGG 180

CGTGACCACC GGCAGATGAG TGTGCTGCAG CAGCATGTGG CTTTCTTCGA CCTGGATGGC 240

GACGGTATCG TTTATCCATG GGAAACTTAT GGAGGACTAC GGGAATTGGG CTTCAACGTG 300

ATTGTTTCGT TCTTTTTGGC GATAGCCATA AACGTTGGTC TAAGCTACCC AACTCTGCCA 360

AGCTGGATAC CATCTCTCCT GTTCCCTATA CACATAAAAA ACATCCACAG GGCTAAGCAC 420

GGCAGCGATA GCTCGACGTA CGACAACGAG GGAAGGTTTA TGCCGGTCAA TTTCGAGAGC 480

ATCTTCAGCA AGAACGCCCG CACGGCGCCG GACAAGCTCA CGTTCGGCGA TATCTGGCGG 540

ATGACCGAAG GCCAAAGGGT GGCGCTCGAC TTGCTTGGGA GGATCGCGAG TAAGGGGGAG 600

TGGATATTGC TCTACGTGCT TGCGAAAGAT GAGGAAGGAT TCCTCAGGAA GGAGGCTGTT 660

CGCCGCTGCT TCGATGGGAG CCTATTCGAG TCGATTGCCC AGCAGAGAAG GGAGGCACAT 720

GAGAAGCAGA AG 732

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 222 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Asp Ala Met Ala Thr Val Ala Pro Tyr Ala Pro Val Thr Tyr His Arg
1 5 10 15

Arg Ala Arg Val Asp Leu Asp Asp Arg Leu Pro Lys Pro Tyr Met Pro
20 25 30

Arg Ala Leu Gln Ala Pro Asp Arg Glu His Pro Tyr Gly Thr Pro Gly
35 40 45

His Lys Asn Tyr Gly Leu Ser Val Leu Gln Gln His Val Ser Phe Phe
50 55 60

Asp Ile Asp Asp Asn Gly Ile Ile Tyr Pro Trp Glu Thr Tyr Ser Gly
65 70 75 80

Leu Arg Met Leu Gly Phe Asn Ile Ile Gly Ser Leu Ile Ile Ala Ala
85 90 95

Val Ile Asn Leu Thr Leu Ser Tyr Ala Thr Leu Pro Gly Trp Leu Pro
100 105 110

Ser Pro Phe Phe Pro Ile Tyr Ile His Asn Ile His Lys Ser Lys His
115 120 125

Gly Ser Asp Ser Lys Thr Tyr Asp Asn Glu Gly Arg Phe Met Pro Val
130 135 140

Asn Leu Glu Leu Ile Phe Ser Lys Tyr Ala Lys Thr Leu Pro Asp Lys
145 150 155 160

Leu Ser Leu Gly Glu Leu Trp Glu Met Thr Glu Gly Asn Arg Asp Ala
165 170 175

Trp Asp Ile Phe Gly Trp Ile Ala Gly Lys Ile Glu Trp Gly Leu Leu
180 185 190

Tyr Leu Leu Ala Arg Asp Glu Glu Gly Phe Leu Ser Lys Glu Ala Ile
195 200 205

Arg Arg Cys Phe Asp Gly Ser Leu Phe Glu Tyr Cys Ala Lys
210 215 220

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 222 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Asp Ala Leu Ser Ser Val Ala Ala Glu Ala Pro Val Thr Arg Glu Arg
1 5 10 15

Pro Val Arg Ala Asp Leu Glu Val Gln Ile Pro Lys Pro Tyr Leu Ala
20 25 30

Arg Ala Leu Val Ala Pro Asp Val Tyr His Pro Glu Gly Thr Glu Gly
35 40 45

Arg Asp His Arg Gln Met Ser Val Leu Gln Gln His Val Ala Phe Phe
50 55 60

Asp Leu Asp Gly Asp Gly Ile Val Tyr Pro Trp Glu Thr Tyr Gly Gly
65 70 75 80

Leu Arg Glu Leu Gly Phe Asn Val Ile Val Ser Phe Phe Leu Ala Ile
85 90 95

Ala Ile Asn Val Gly Leu Ser Tyr Pro Thr Leu Pro Ser Trp Ile Pro
100 105 110

Ser Leu Leu Phe Pro Ile His Ile Lys Asn Ile His Arg Ala Lys His
115 120 125

Gly Ser Asp Ser Ser Thr Tyr Asp Asn Glu Gly Arg Phe Met Pro Val
130 135 140

Asn Phe Glu Ser Ile Phe Ser Lys Asn Ala Arg Thr Ala Pro Asp Lys
145 150 155 160

Leu Thr Phe Gly Asp Ile Trp Arg Met Thr Glu Gly Gln Arg Val Ala
165 170 175

Leu Asp Leu Leu Gly Arg Ile Ala Ser Lys Gly Glu Trp Ile Leu Leu
180 185 190

Tyr Val Leu Ala Lys Asp Glu Glu Gly Phe Leu Arg Lys Glu Ala Val
195 200 205

Arg Arg Cys Phe Asp Gly Ser Leu Phe Glu Ser Ile Ala Gln
210 215 220

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 256 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTCTTCAACA GCATGTCTCC TTCTTCGATA TCGATGATAA TGGCATCATT TACCCTTGGG 60

AGACCTACTC TGGACTGCGA ATGCTTGGTT TCAATATCAT TGGGTCGCTT ATAATAGCCG 120

CTGTTATCAA CCTGACCCTT AGCTATGCCA CTCTTCCGGG GTGGTTACCT TCACCTTTCT 180

TCCCTATATA CATACACAAC ATACACAAGT CAAAGCATGG AAGTGATTCA AAAACATATG 240

ACAATGAAGG AAGGTT 256

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 257 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TTCTTGCAGA GACATGTCGC TTTTTTCGAT AGGAACAAAG ATGGTATCGT TTATCCCTCG 60

GAGACATTTC AAGGATTTAG AGCAATTGGG TGTGGATATT TGTTGTCAGC AGTCGCTTCT 120

GTGTTCATAA ACATAGGTCT CAGCAGCAAA ACTCGTCCGG GTAAAGGATT CTCTATCTGG 180

TTTCCTATAG AGGTTAAGAA TATTCACCTT GCCAAACACG GAAGCGATTC AGGCGTTTAC 240

GACAAAGATG GACGGTT 257

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 273 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCACCGGCAG ATGAGTGTGC TGCAGCAGCA TGTGGCTTTC TTCGACCTGG ATGGCGACGG 60

TATCGTTTAT CCATGGGAAA CTTATGGAGG ACTACGGGAA TTGGGCTTCA ACGTGATTGT 120

TTCGTTCTTT TTGGCGATAG CCATAAACGT TGGTCTAAGC TACCCAACTC TGCCAAGCTG 180

GATACCATCT CTCCTGTTCC CTATACACAT AAAAAACATC CACAGGGCTA AGCACGGCAG 240

CGATAGCTCG ACGTACGACA ACGAGGGAAG GTT 273

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 272 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCAGAAGAAG ATAATTTCTT GCAGAGACAT GTCGCTTTTT TCGATAGGAA CAAAGATGGT 60

ATCGTTTATC CCTCGGAGAC ATTTCAAGGA TTTAGAGCAA TTGGGTGTGG ATATTTGTTG 120

TCAGCAGTCG CTTCTGTGTT CATAAACATA GGTCTCAGCA GCAAAACTCG TCCGGGTAAA 180

GGATTCTCTA TCTGGTTTCC TATAGAGGTT AAGAATATTC ACCTTGCCAA ACACGGAAGC 240

GATTCAGGCG TTTACGACAA AGATGGACGG TT 272

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1211 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CGATCCACCC CATGACTCGA ATGATGACTC CAGCCACCAT CAATTCCCTG AAGTGCCACA 60

ACACCCTCTA CCTCCCAGGT TTTATGACAA TCCGACCAAC GATTATCCCG CAGATGTCCC 120

ACCTCCACCA CCGTCTTCTT ACCCTTCCAA CGATCATCTT CCCCCTCCCA CAGGACCATC 180

AGACTCCCCT TACCCGCATC CTTACAGTCA TCAACCATAC CACCAAGACC CGCCAAAACA 240

CATGCCGCCA CCGCAAAACT ACTCATCTCA TGAGCCTTCT CCAAATTCTC TCCCTAATTT 300

CCAATCTTAT CCTAGCTTTA GTGAGAGCAG CCTCCCATCC ACTTCTCCCC ACTACCCTTC 360

TCACTACCAA AACCCAGAAC CTTACTATTC TTCTCCGCAC TCTGCACCTG CTCCTTCTTC 420

CACAAGCTTC TGCTCTGCTC CTCCTCCTCC ACCTTACTCA TCAAACGGGC GTATCAATAT 480

TGCTCCCGTG CTAGATCCTG CACCGAGTTC AGCTCAGAAG TACCATTACG ATAGCAGCTA 540

CCAGCCAGGG CCTGAGAAGG TTGCAGAGGC ACTCAAGGCT GCTAGATTCG CTGTGGGAGC 600

TTTGGCTTTT GATGAAGTCT CGACTGCTGT AGAACATCTC AAGAAGTCAC TTGAGTTGCT 660

AACAAATCCA TCGGCCGGTG CCGGTCACTG AATTTTATAT CTAATCTATG ACACTTGGGG 720

TTGATGTTAG TGCGTGTGTG TGTTCTCACC ACATTTGTGG GTTTGTTTAT TAACTTTTCA 780

GGCTCAGACT TCGTTTACAA AGAAAATTTG TGTGAATTAT TCTTATTATC ATAAAATTTT 840

CCTTGCAACT TCGTGTACAT TCATACATAC ATAGGCAATG GAGTTCCTCT TCAGTCTTCA 900

CGTAAAGAGC GAGTGTGGGA CACGCACTCA TGTAGCGGGT GGTGTTAGTA CTCGAGGTTG 960

GGCCTATATA AAAGCCCATA GAGGCCCGAA TTACTGAATT TAGCAGACAA GAATAGAAAG 1020

AGTGATGAAA CATGGAAGAA AACGTGTCTC TAGAGTCATG TCAAGTGTAA GACAGAGGAA 1080

GAGAGAAGAG ATGTGCGTCA AAGACAAGGA AAGAGAGATG TCAATCGCTG CTTTCGTCGG 1140

CGCGTGCATG TCCGCCACGC ACATCAATCA AATCGATTCT TATTATTATT ACCTCATTAT 1200

ACTCTTTACT C 1211

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1486 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TGCATGGGAA GTAATTTTAA TTAACCTATG TTTTAAACAT TTACATTATT TGGAATTAAT 60

ATTATATATA CACTATTCGA TTTTGTTTTC CTTCAATGTA ACATTACTCT GGCAAAAGTA 120

TTTATCGTAT AATATCTTTT ATTATAAATT TTTGATGTTT TAAAGATTAG TTTATCTCTT 180

TTGACCAAAA AGAAAGGAAA AGGGATTAGA TTTATCTCTA TGTGAACTTG ATTATACGAG 240

TTCGGATAAT CGGATCTCAA TGTGATATCC ATATTTCTTG CAAGACATAT CTCTCGTACA 300

CCTTTTATAT TTATATCCCG CAATCGTGAC AACTCTTAAT CATTCACTAC ATAATATTTC 360

CAACAACATT AAAAGATATT TATCTTAATT CTCTTTTCCT TAACACTAAC AAAGTAGCAT 420

GTCCATATAT ACTTTCGTTT TTTGAGCATG AGAAAATAGA TTTAACTTTA TAAGTTATAA 480

CCATTGTTTC AAATTAATGC AGATTCGAGT AATAATAATT TGAGATGCAA TAATGGTTGT 540

GTCATATCTT GATTGCTAAA CTTGATACCG CCATACCGGT AACGTGAAGG GAGAGCTTCC 600

AATTTGTATG CAAGCCTACA TCTGACCCAA TTGTTGGCCC AATATTAACC AACACCCACA 660

CTAAAAAAAA TACTATGGAG GGAGTAATCT ACATGCCTAC ATTCCAAAGC AGGCAATATC 720

GTTTTTTCAT GTCTGAAAAC GCAATTTTTT TTTCTAATTG TTAAGTTGGT TCAAAAGAAA 780

TGAACATGGG TAATAATAAA AATGATGTAT TTGTTTGCAA ACAGCAGTTC TCACTTGTCT 840

CTCTCTATAT GATGAAAGAC AATGTTGTAA TCTTTATAGG TTTCAATATA GCGGGTATAC 900

TTGGTGACAT AAAGCGTTAT GAAATTTTAA GCAGTAAATA GGAAATGATA AATGATTATT 960

AAATTCGTTA TTAAAAATGT AAGAAGGAAT AGTACAATAT AGAACGGTAA AAAAAATGGC 1020

AAACCATTTA CTTCAATAAG AAAGGTTAGC AACCACACTC AGCAAATGGG ACACATAGGA 1080

TCCGACGTGG TTTATATTAT AGTAGTCTGA TATTGTAGAG TCAATGGGTA TATTTGTCTT 1140

TTTCAAAGAC TCAGTTCCAT TGAAGCGTAG GTTACTTCTT TAAACAAGAC TCTGTTTTGA 1200

ATGATATTGT AAAGTTAAGG GGTACGTTTG TCTTTTTCAG GACAAAGCGA GACCATAGAT 1260

GACGTGTCAA CTGCTAATTT TCAAAAACTC GGTCTACAAA CCATAACCAA ACTTATTTAT 1320

TCAATTATTT CCGTCAAAAA AATATAATTT TCTTTTTGCA TCTCAATGGA TTGATTCCAT 1380

GTGCCAAGTG TTGGTGTTCA TGAGAAAATT AGTCGCAGCT GATGACAACA AACATCAAGC 1440

ATTTATAATT TATATAACAC TCACGAGTGC CTCTTTCTTT GGATCC 1486

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 62 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTTACTCTAA GACAAACACA TACATTTGCA CTCAGTCTAG AGACAAAGAG AGAGAGCCAT 60

GG 62

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AGTGCCTCTT TCTTTATCTA CCTCGTCTCC TAATCACAAA CACACACAAA TCTCTGAAGT 60

ACCATG 66

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1266 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CGATCCACCC CATGACTCGA ATGATGACTC CAGCCACCAT CAATTCCCTG AAGTGCCACA 60

ACACCCTCTA CCTCCCAGGT TTTATGACAA TCCGACCAAC GATTATCCCG CAGATGTCCC 120

ACCTCCACCA CCGTCTTCTT ACCCTTCCAA CGATCATCTT CCCCCTCCCA CAGGACCATC 180

AGACTCCCCT TACCCGCATC CTTACAGTCA TCAACCATAC CACCAAGACC CGCCAAAACA 240

CATGCCGCCA CCGCAAAACT ACTCATCTCA TGAGCCTTCT CCAAATTCTC TCCCTAATTT 300

CCAATCTTAT CCTAGCTTTA GTGAGAGCAG CCTCCCATCC ACTTCTCCCC ACTACCCTTC 360

TCACTACCAA AACCCAGAAC CTTACTATTC TTCTCCGCAC TCTGCACCTG CTCCTTCTTC 420

CACAAGCTTC TGCTCTGCTC CTCCTCCTCC ACCTTACTCA TCAAACGGGC GTATCAATAT 480

TGCTCCCGTG CTAGATCCTG CACCGAGTTC AGCTCAGAAG TACCATTACG ATAGCAGCTA 540

CCAGCCAGGG CCTGAGAAGG TTGCAGAGGC ACTCAAGGCT GCTAGATTCG CTGTGGGAGC 600

TTTGGCTTTT GATGAAGTCT CGACTGCTGT AGAACATCTC AAGAAGTCAC TTGAGTTGCT 660

AACAAATCCA TCGGCCGGTG CCGGTCACTG AATTTTATAT CTAATCTATG ACACTTGGGG 720

TTGATGTTAG TGCGTGTGTG TGTTCTCACC ACATTTGTGG GTTTGTTTAT TAACTTTTCA 780

GGCTCAGACT TCGTTTACAA AGAAAATTTG TGTGAATTAT TCTTATTATC ATAAAATTTT 840

CCTTGCAACT TCGTGTACAT TCATACATAC ATAGGCAATG GAGTTCCTCT TCAGTCTTCA 900

CGTAAAGAGC GAGTGTGGGA CACGCACTCA TGTAGCGGGT GGTGTTAGTA CTCGAGGTTG 960

GGCCTATATA AAAGCCCATA GAGGCCCGAA TTACTGAATT TAGCAGACAA GAATAGAAAG 1020

AGTGATGAAA CATGGAAGAA AACGTGTCTC TAGAGTCATG TCAAGTGTAA GACAGAGGAA 1080

GAGAGAAGAG ATGTGCGTCA AAGACAAGGA AAGAGAGATG TCAATCGCTG CTTTCGTCGG 1140

CGCGTGCATG TCCGCCACGC ACATCAATCA AATCGATTCT TATTATTATT ACCTCATTAT 1200

ACTCTTTACT CTAAGACAAA CACATACATT TGCACTCAGT CTAGAGACAA AGAGAGAGAG 1260

CCATGG 1266

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1532 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TGCATGGGAA GTAATTTTAA TTAACCTATG TTTTAAACAT TTACATTATT TGGAATTAAT 60

ATTATATATA CACTATTCGA TTTTGTTTTC CTTCAATGTA ACATTACTCT GGCAAAAGTA 120

TTTATCGTAT AATATCTTTT ATTATAAATT TTTGATGTTT TAAAGATTAG TTTATCTCTT 180

TTGACCAAAA AGAAAGGAAA AGGGATTAGA TTTATCTCTA TGTGAACTTG ATTATACGAG 240

TTCGGATAAT CGGATCTCAA TGTGATATCC ATATTTCTTG CAAGACATAT CTCTCGTACA 300

CCTTTTATAT TTATATCCCG CAATCGTGAC AACTCTTAAT CATTCACTAC ATAATATTTC 360

CAACAACATT AAAAGATATT TATCTTAATT CTCTTTTCCT TAACACTAAC AAAGTAGCAT 420

GTCCATATAT ACTTTCGTTT TTTGAGCATG AGAAAATAGA TTTAACTTTA TAAGTTATAA 480

CCATTGTTTC AAATTAATGC AGATTCGAGT AATAATAATT TGAGATGCAA TAATGGTTGT 540

GTCATATCTT GATTGCTAAA CTTGATACCG CCATACCGGT AACGTGAAGG GAGAGCTTCC 600

AATTTGTATG CAAGCCTACA TCTGACCCAA TTGTTGGCCC AATATTAACC AACACCCACA 660

CTAAAAAAAA TACTATGGAG GGAGTAATCT ACATGCCTAC ATTCCAAAGC AGGCAATATC 720

GTTTTTTCAT GTCTGAAAAC GCAATTTTTT TTTCTAATTG TTAAGTTGGT TCAAAAGAAA 780

TGAACATGGG TAATAATAAA AATGATGTAT TTGTTTGCAA ACAGCAGTTC TCACTTGTCT 840

CTCTCTATAT GATGAAAGAC AATGTTGTAA TCTTTATAGG TTTCAATATA GCGGGTATAC 900

TTGGTGACAT AAAGCGTTAT GAAATTTTAA GCAGTAAATA GGAAATGATA AATGATTATT 960

AAATTCGTTA TTAAAAATGT AAGAAGGAAT AGTACAATAT AGAACGGTAA AAAAAATGGC 1020

AAACCATTTA CTTCAATAAG AAAGGTTAGC AACCACACTC AGCAAATGGG ACACATAGGA 1080

TCCGACGTGG TTTATATTAT AGTAGTCTGA TATTGTAGAG TCAATGGGTA TATTTGTCTT 1140

TTTCAAAGAC TCAGTTCCAT TGAAGCGTAG GTTACTTCTT TAAACAAGAC TCTGTTTTGA 1200

ATGATATTGT AAAGTTAAGG GGTACGTTTG TCTTTTTCAG GACAAAGCGA GACCATAGAT 1260

GACGTGTCAA CTGCTAATTT TCAAAAACTC GGTCTACAAA CCATAACCAA ACTTATTTAT 1320

TCAATTATTT CCGTCAAAAA AATATAATTT TCTTTTTGCA TCTCAATGGA TTGATTCCAT 1380

GTGCCAAGTG TTGGTGTTCA TGAGAAAATT AGTCGCAGCT GATGACAACA AACATCAAGC 1440

ATTTATAATT TATATAACAC TCACGAGTGC CTCTTTCTTT ATCTACCTCG TCTCCTAATC 1500

ACAAACACAC ACAAATCTCT GAAGTACCAT GG 1532

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TTATTATTAC CTC 13

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GAAGTCTATC ATCC 14

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CACTCACGAG TGCCTC 16

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ACAAGAAGAA CCTGG 15

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

ACCGAATTCA TGGCATTCGA CCTCAGCTCT 30

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGTGAGCTCT CACTAATTTC CAAGCCTTGA 30

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATTAACCCTC ACTAAAG 17

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

AATACGACTC ACTATAG 17

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CATGCCATGG CTCTCTCTCT TTGTCTCTAG ACTG 34

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CTAGCCATGG TACTTCAGAG ATTTGTGTG 29

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1248 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

AAGCTCGATC CACCCCATGA CTCGAATGAT GACTCCAGCC ACCATCAATT CCCTGAAGTG 60

CCACAACACC CTCTACCTCC CAGGTTTTAT GACAATCCGA CCAACGATTA TCCCGCAGAT 120

GTCCCACCTC CACCACCGTC TTCTTACCCT TCCAACGATC ATCTTCCCCC TCCCACAGGA 180

CCATCAGACT CCCCTTACCC GCATCCTTAC AGTCATCAAC CATACCACCA AGACCCGCCA 240

AAACACATGC CGCCACCGCA AAACTACTCA TCTCATGAGC CTTCTCCAAA TTCTCTCCCT 300

AATTTCCAAT CTTATCCTAG CTTTAGTGAG AGCAGCCTCC CATCCACTTC TCCCCACTAC 360

CCTTCTCACT ACCAAAACCC AGAACCTTAC TATTCTTCTC CGCACTCTGC ACCTGCTCCT 420

TCTTCCACAA GCTTCTGCTC TGCTCCTCCT CCTCCACCTT ACTCATCAAA CGGGCGTATC 480

AATATTGCTC CCGTGCTAGA TCCTGCACCG AGTTCAGCTC AGAAGTACCA TTACGATAGC 540

AGCTACCAGC CAGGGCCTGA GAAGGTTGCA GAGGCACTCA AGGCTGCTAG ATTCGCTGTG 600

GGAGCTTTGG CTTTTGATGA AGTCTCGACT GCTGTAGAAC ATCTCAAGAA GTCACTTGAG 660

TTGCTAACAA ATCCATCGGC CGGTGCCGGT CACTGAATTT TATATCTAAT CTATGACACT 720

TGGGGTTGAT GTTAGTGCGT GTGTGTGTTC TCACCACATT TGTGGGTTTG TTTATTAACT 780

TTTCAGGCTC AGACTTCGTT TACAAAGAAA ATTTGTGTGA ATTATTCTTA TTATCATAAA 840

ATTTTCCTTG CAACTTCGTG TACATTCATA CATACATAGG CAATGGAGTT CCTCTTCAGT 900

CTTCACGTAA AGAGCGAGTG TGGGACACGC ACTCATGTAG CGGGTGGTGT TAGTACTCGA 960

GGTTGGGCCT ATATAAAAGC CCATAGAGGC CCGAATTACT GAATTTAGCA GACAAGAATA 1020

GAAAGAGTGA TGAAACATGG AAGAAAACGT GTCTCTAGAG TCATGTCAAG TGTAAGACAG 1080

AGGAAGAGAG AAGAGATGTG CGTCAAAGAC AAGGAAAGAG AGATGTCAAT CGCTGCTTTC 1140

GTCGGCGCGT GCATGTCCGC CACGCACATC AATCAAATCG ATTCTTATTA TTATTACCTC 1200

ATTATACTCT TTACTCCCTA GGCGCGATCC CCGGGTGGTC AGTTCCTT 1248

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1307 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GGATCCACTA GTTCTAGAGC GGCCGCCACC GCGGTGGAGC TCGATCCACC CCATGACTCG 60

AATGATGACT CCAGCCACCA TCAATTCCCT GAAGTGCCAC AACACCCTCT ACCTCCCAGG 120

TTTTATGACA ATCCGACCAA CGATTATCCC GCAGATGTCC CACCTCCACC ACCGTCTTCT 180

TACCCTTCCA ACGATCATCT TCCCCCTCCC ACAGGACCAT CAGACTCCCC TTACCCGCAT 240

CCTTACAGTC ATCAACCATA CCACCAAGAC CCGCCAAAAC ACATGCCGCC ACCGCAAAAC 300

TACTCATCTC ATGAGCCTTC TCCAAATTCT CTCCCTAATT TCCAATCTTA TCCTAGCTTT 360

AGTGAGAGCA GCCTCCCATC CACTTCTCCC CACTACCCTT CTCACTACCA AAACCCAGAA 420

CCTTACTATT CTTCTCCGCA CTCTGCACCT GCTCCTTCTT CCACAAGCTT CTGCTCTGCT 480

CCTCCTCCTC CACCTTACTC ATCAAACGGG CGTATCAATA TTGCTCCCGT GCTAGATCCT 540

GCACCGAGTT CAGCTCAGAA GTACCATTAC GATAGCAGCT ACCAGCCAGG GCCTGAGAAG 600

GTTGCAGAGG CACTCAAGGC TGCTAGATTC GCTGTGGGAG CTTTGGCTTT TGATGAAGTC 660

TCGACTGCTG TAGAACATCT CAAGAAGTCA CTTGAGTTGC TAACAAATCC ATCGGCCGGT 720

GCCGGTCACT GAATTTTATA TCTAATCTAT GACACTTGGG GTTGATGTTA GTGCGTGTGT 780

GTGTTCTCAC CACATTTGTG GGTTTGTTTA TTAACTTTTC AGGCTCAGAC TTCGTTTACA 840

AAGAAAATTT GTGTGAATTA TTCTTATTAT CATAAAATTT TCCTTGCAAC TTCGTGTACA 900

TTCATACATA CATAGGCAAT GGAGTTCCTC TTCAGTCTTC ACGTAAAGAG CGAGTGTGGG 960

ACACGCACTC ATGTAGCGGG TGGTGTTAGT ACTCGAGGTT GGGCCTATAT AAAAGCCCAT 1020

AGAGGCCCGA ATTACTGAAT TTAGCAGACA AGAATAGAAA GAGTGATGAA ACATGGAAGA 1080

AAACGTGTCT CTAGAGTCAT GTCAAGTGTA AGACAGAGGA AGAGAGAAGA GATGTGCGTC 1140

AAAGACAAGG AAAGAGAGAT GTCAATCGCT GCTTTCGTCG GCGCGTGCAT GTCCGCCACG 1200

CACATCAATC AAATCGATTC TTATTATTAT TACCTCATTA TACTCTTTAC TCTAAGACAA 1260

ACACATACAT TTGCACTCAG TCTAGAGACA AAGAGAGAGA GCCATGG 1307

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1511 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TCTAGATGCA TGGGAAGTAA TTTTAATTAA CCTATGTTTT AAACATTTAC ATTATTTGGA 60

ATTAATATTA TATATACACT ATTCGATTTT GTTTTCCTTC AATGTAACAT TACTCTGGCA 120

AAAGTATTTA TCGTATAATA TCTTTTATTA TAAATTTTTG ATGTTTTAAA GATTAGTTTA 180

TCTCTTTTGA CCAAAAAGAA AGGAAAAGGG ATTAGATTTA TCTCTATGTG AACTTGATTA 240

TACGAGTTCG GATAATCGGA TCTCAATGTG ATATCCATAT TTCTTGCAAG ACATATCTCT 300

CGTACACCTT TTATATTTAT ATCCCGCAAT CGTGACAACT CTTAATCATT CACTACATAA 360

TATTTCCAAC AACATTAAAA GATATTTATC TTAATTCTCT TTTCCTTAAC ACTAACAAAG 420

TAGCATGTCC ATATATACTT TCGTTTTTTG AGCATGAGAA AATAGATTTA ACTTTATAAG 480

TTATAACCAT TGTTTCAAAT TAATGCAGAT TCGAGTAATA ATAATTTGAG ATGCAATAAT 540

GGTTGTGTCA TATCTTGATT GCTAAACTTG ATACCGCCAT ACCGGTAACG TGAAGGGAGA 600

GCTTCCAATT TGTATGCAAG CCTACATCTG ACCCAATTGT TGGCCCAATA TTAACCAACA 660

CCCACACTAA AAAAAATACT ATGGAGGGAG TAATCTACAT GCCTACATTC CAAAGCAGGC 720

AATATCGTTT TTTCATGTCT GAAAACGCAA TTTTTTTTTC TAATTGTTAA GTTGGTTCAA 780

AAGAAATGAA CATGGGTAAT AATAAAAATG ATGTATTTGT TTGCAAACAG CAGTTCTCAC 840

TTGTCTCTCT CTATATGATG AAAGACAATG TTGTAATCTT TATAGGTTTC AATATAGCGG 900

GTATACTTGG TGACATAAAG CGTTATGAAA TTTTAAGCAG TAAATAGGAA ATGATAAATG 960

ATTATTAAAT TCGTTATTAA AAATGTAAGA AGGAATAGTA CAATATAGAA CGGTAAAAAA 1020

AATGGCAAAC CATTTACTTC AATAAGAAAG GTTAGCAACC ACACTCAGCA AATGGGACAC 1080

ATAGGATCCG ACGTGGTTTA TATTATAGTA GTCTGATATT GTAGAGTCAA TGGGTATATT 1140

TGTCTTTTTC AAAGACTCAG TTCCATTGAA GCGTAGGTTA CTTCTTTAAA CAAGACTCTG 1200

TTTTGAATGA TATTGTAAAG TTAAGGGGTA CGTTTGTCTT TTTCAGGACA AAGCGAGACC 1260

ATAGATGACG TGTCAACTGC TAATTTTCAA AAACTCGGTC TACAAACCAT AACCAAACTT 1320

ATTTATTCAA TTATTTCCGT CAAAAAAATA TAATTTTCTT TTTGCATCTC AATGGATTGA 1380

TTCCATGTGC CAAGTGTTGG TGTTCATGAG AAAATTAGTC GCAGCTGATG ACAACAAACA 1440

TCAAGCATTT ATAATTTATA TAACACTCAC GAGTGCCTCT TTCTTTGGAT CCGCGGGGTG 1500

GTCAGTTCCT T 1511

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1538 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TCTAGATGCA TGGGAAGTAA TTTTAATTAA CCTATGTTTT AAACATTTAC ATTATTTGGA 60

ATTAATATTA TATATACACT ATTCGATTTT GTTTTCCTTC AATGTAACAT TACTCTGGCA 120

AAAGTATTTA TCGTATAATA TCTTTTATTA TAAATTTTTG ATGTTTTAAA GATTAGTTTA 180

TCTCTTTTGA CCAAAAAGAA AGGAAAAGGG ATTAGATTTA TCTCTATGTG AACTTGATTA 240

TACGAGTTCG GATAATCGGA TCTCAATGTG ATATCCATAT TTCTTGCAAG ACATATCTCT 300

CGTACACCTT TTATATTTAT ATCCCGCAAT CGTGACAACT CTTAATCATT CACTACATAA 360

TATTTCCAAC AACATTAAAA GATATTTATC TTAATTCTCT TTTCCTTAAC ACTAACAAAG 420

TAGCATGTCC ATATATACTT TCGTTTTTTG AGCATGAGAA AATAGATTTA ACTTTATAAG 480

TTATAACCAT TGTTTCAAAT TAATGCAGAT TCGAGTAATA ATAATTTGAG ATGCAATAAT 540

GGTTGTGTCA TATCTTGATT GCTAAACTTG ATACCGCCAT ACCGGTAACG TGAAGGGAGA 600

GCTTCCAATT TGTATGCAAG CCTACATCTG ACCCAATTGT TGGCCCAATA TTAACCAACA 660

CCCACACTAA AAAAAATACT ATGGAGGGAG TAATCTACAT GCCTACATTC CAAAGCAGGC 720

AATATCGTTT TTTCATGTCT GAAAACGCAA TTTTTTTTTC TAATTGTTAA GTTGGTTCAA 780

AAGAAATGAA CATGGGTAAT AATAAAAATG ATGTATTTGT TTGCAAACAG CAGTTCTCAC 840

TTGTCTCTCT CTATATGATG AAAGACAATG TTGTAATCTT TATAGGTTTC AATATAGCGG 900

GTATACTTGG TGACATAAAG CGTTATGAAA TTTTAAGCAG TAAATAGGAA ATGATAAATG 960

ATTATTAAAT TCGTTATTAA AAATGTAAGA AGGAATAGTA CAATATAGAA CGGTAAAAAA 1020

AATGGCAAAC CATTTACTTC AATAAGAAAG GTTAGCAACC ACACTCAGCA AATGGGACAC 1080

ATAGGATCCG ACGTGGTTTA TATTATAGTA GTCTGATATT GTAGAGTCAA TGGGTATATT 1140

TGTCTTTTTC AAAGACTCAG TTCCATTGAA GCGTAGGTTA CTTCTTTAAA CAAGACTCTG 1200

TTTTGAATGA TATTGTAAAG TTAAGGGGTA CGTTTGTCTT TTTCAGGACA AAGCGAGACC 1260

ATAGATGACG TGTCAACTGC TAATTTTCAA AAACTCGGTC TACAAACCAT AACCAAACTT 1320

ATTTATTCAA TTATTTCCGT CAAAAAAATA TAATTTTCTT TTTGCATCTC AATGGATTGA 1380

TTCCATGTGC CAAGTGTTGG TGTTCATGAG AAAATTAGTC GCAGCTGATG ACAACAAACA 1440

TCAAGCATTT ATAATTTATA TAACACTCAC GAGTGCCTCT TTCTTTATCT ACCTCGTCTC 1500

CTAATCACAA ACACACACAA ATCTCTGAAG TACCATGG 1538

INTERNATIONAL SEARCH REPORT

Int. tional Application No
PCT/EP 98/06978

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/29 C12N9/02 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NUCCIO, M.L., ET AL. : "characterization of two novel seed-specific cDNAs identified in immature Arabidopsis thaliana seeds" SUPPLEMENTS TO PLANT PHYSIOLOGY, vol. 111, no. 2, June 1996, page 158 XP002095424	1-22, 26-35
Y	see the whole document	23-25
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 March 1999	18/03/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int'l tional Application No
PCT/EP 98/06978

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHONGSEN, L.: "ISOLATION AND CHARACTERIZATION OF ARABIDOPSIS EMBRYO-SPECIFIC GENES (VIRTUAL SUBTRACTION, DNA BINDING SITES, GENE ISOLATION)" DISSERATION TEXAS A&M UNIVERSITY,May 1997, XP002075900	1-3,6,8, 15-21, 26-35
Y	page 33, Table 2-1; page 107-128; Fig. 5-4-;page 134	23-25
X	PLANT ET AL: "Regulation of an Arabidopsis oleosin gene promoter in transgenic Brassica napus" PLANT MOLECULAR BIOLOGY, no. 25, 1994, page 193 193 XP002075902 abstract; page 194; Fig.1; page 197; Fig. 4; page 199,201; page 202, left column; 203	1-3,11, 12, 15-20, 26-35
X	WO 92 18634 A (UNILEVER PLC ;UNILEVER NV (NL)) 29 October 1992 page 8,13,20,21	1,6, 15-35
Y	BEREMAND ET AL: "Production of gamma-linolenic acid by transgenic plants expressing cyanobacterial or plant delta6-desaturase genes" PHYSIOLOGY, BIOCHEMISTRY AND MOLECULAR BIOLOGY OF PLANT LIPIDS,1997, page 351 351 XP002076486 see the whole document	23-25
A	FRANDSEN,G., ET AL. : "novel plant Ca2+-binding protein expressed in response to abscisic acid and osmotic stress" THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 1, January 1996, pages 343-348, XP002095425 cited in the application see the whole document	1-35
A	RAYNAL, M., ET AL. : "the Arabidopsis thaliana transcribed genome: the GDR cDNA program" EMBL SEQUENCE DATA LIBRARY, 1 November 1993, XP002095426 heidelberg, germany cited in the application accession no. Z27053	1-35

-/--

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int: tional Application No
PCT/EP 98/06978

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RAYNAL,M., ET AL.: "the Arabidopsis thaliana transcribed genome: the GDR cDNA program" EMBL SEQUENCE DATA LIBRARY, 9 February 1994, XP002095427 heidelberg, germany cited in the application accession no. Z29900	1-35
P,X	NUCCIO,M.L. AND THOMAS,T.L.: "a novel embryo-specific gene in Arabidopsis thaliana" EMBL SEQUENCE DATA LIBRARY,22 July 1998, XP002095428 heidelberg, germany accession no. AF067857	1,5,6,8, 11,12
P,X	NUCCIO, M.L. AND THOMAS, T.L.: "novel Arabidopsis embryo-specific gene" EMBL SEQUENCE DATA LIBRARY,22 July 1998, XP002095429 heidelberg,germany accession no. AF067858	1,3,6,8, 11,12

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/EP 98/06978

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Due to the fact that no amino acid sequence was filed for the cDNA clone AtS3, a complete search could not be carried out for technical content concerning said clone.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: claims 4, 9, 13 completely; claims 1, 2, 3, 6, 7, 8, 11, 12, 15-35 partially

   Seed-specific regulatory sequences from the clone AtS1 isolated from Arabidopsis thaliana utilized for the expression of heterologous genes in transgenic plants.

2. Claims: claims 5, 10, 14 completely; claims 1, 2, 3, 6,7, 8, 11, 12, 15-35 partially

   Seed-specific regulatory sequences from the clone AtS3 isolated from Arabidopsis thaliana utilized for the expression of heterologous genes in transgenic plants.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ..tional Application No
PCT/EP 98/06978

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9218634 A	29-10-1992	AU 669478 B	13-06-1996
		AU 1468092 A	17-11-1992
		CA 2106960 A	10-10-1992
		EP 0580649 A	02-02-1994
		JP 6506584 T	28-07-1994
		US 5767363 A	16-06-1998
		ZA 9202592 A	11-10-1993